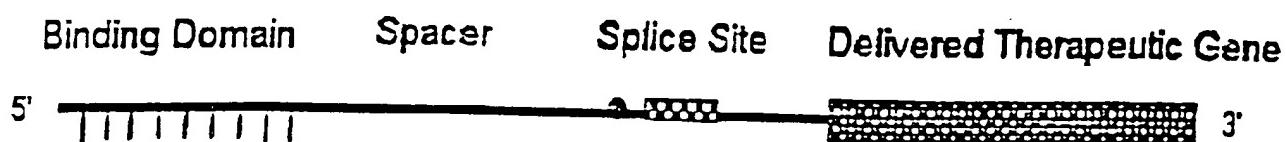
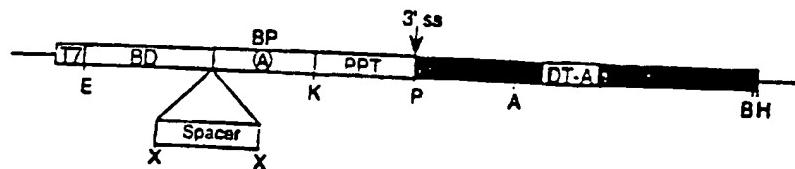


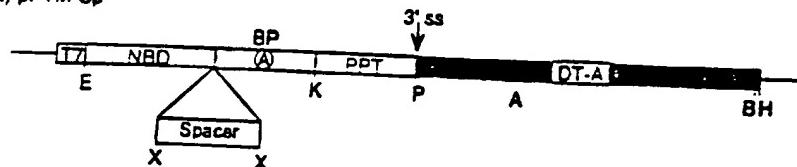
FIGURE 1A



(B) (1) pPTM+Sp



(2) pPTM-Sp



(C)

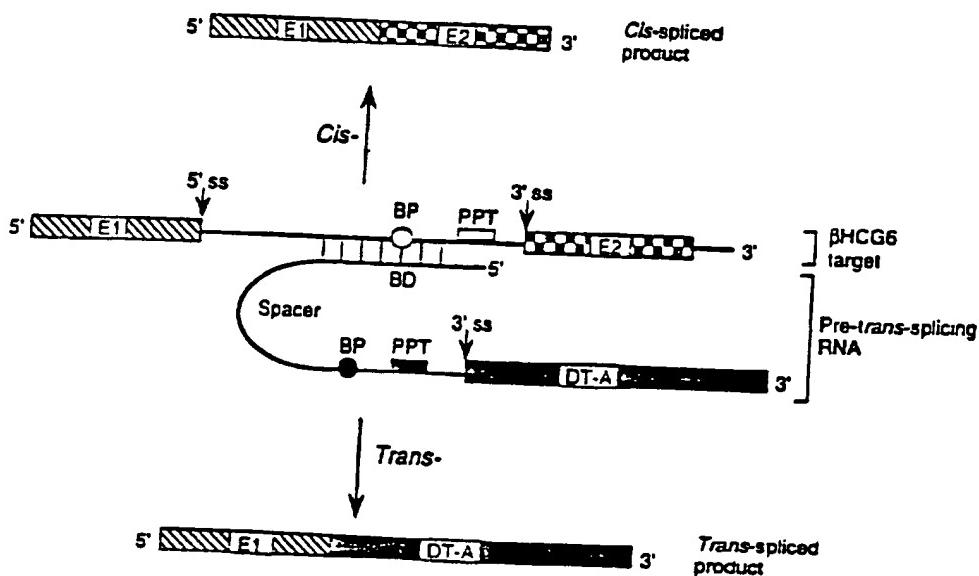
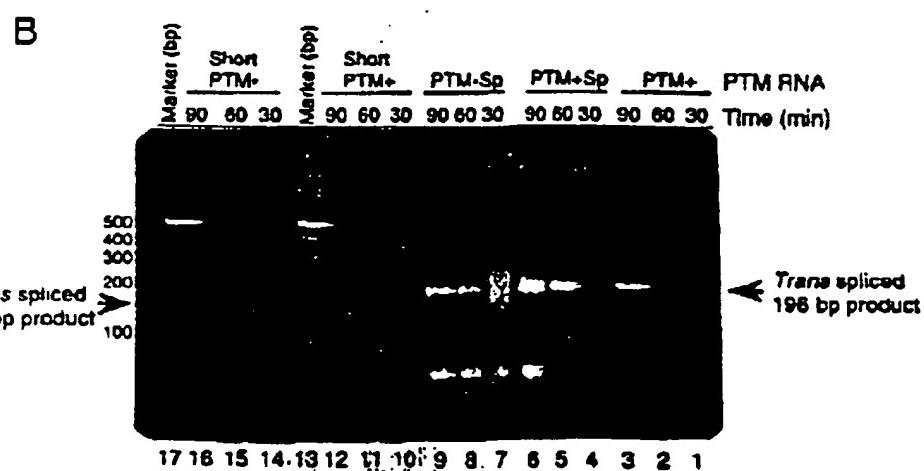
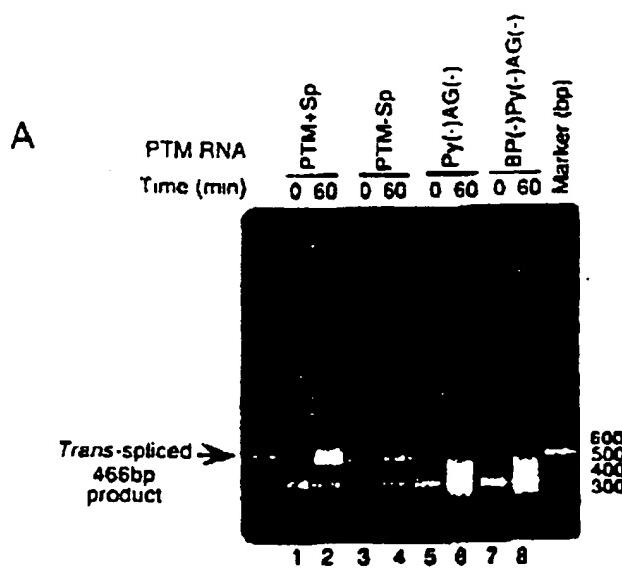
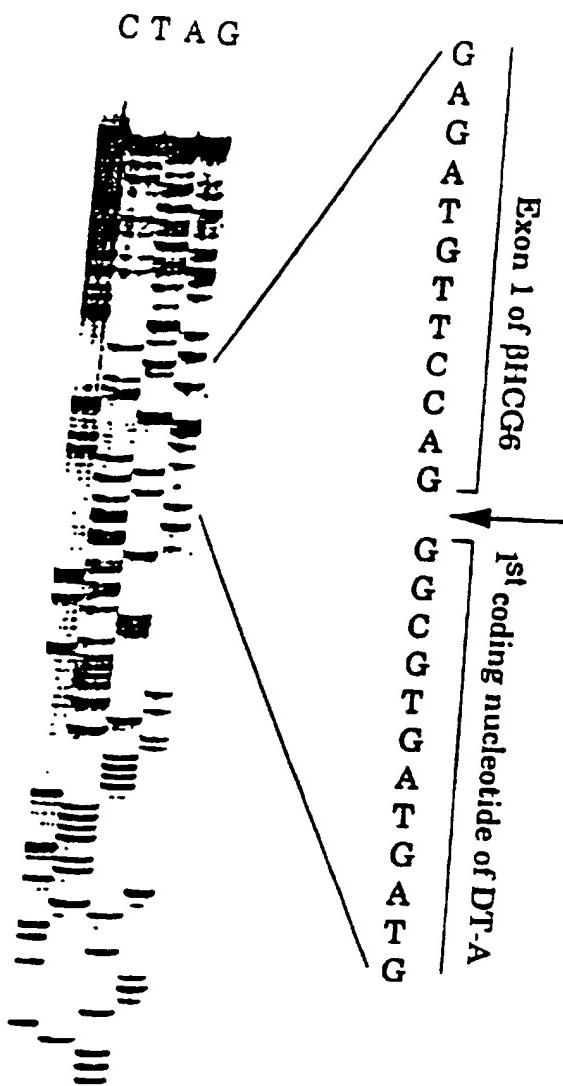


Figure 1 B-C

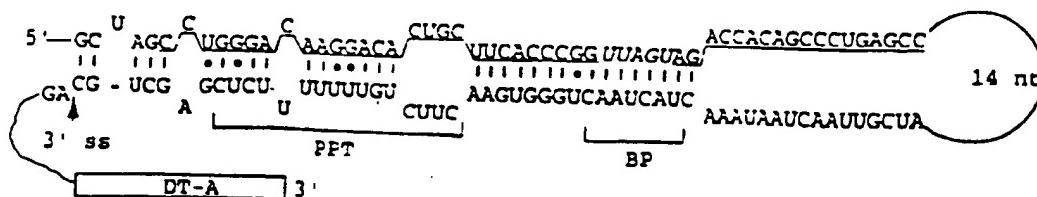




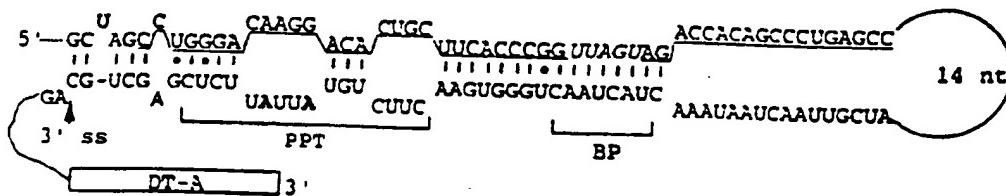
(A)

51504B-A
(Sheet 5 Of 58)

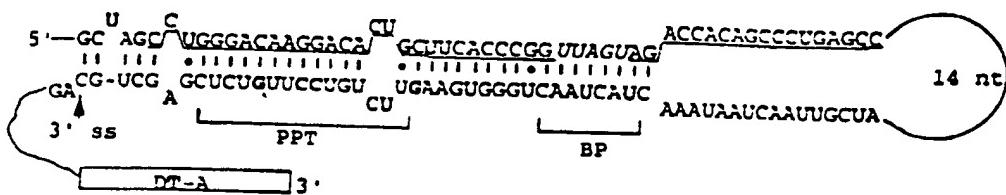
1. PTM+SF+



2. PTM+SF-Py1:



3. PTM+SF-Py2:



(B)

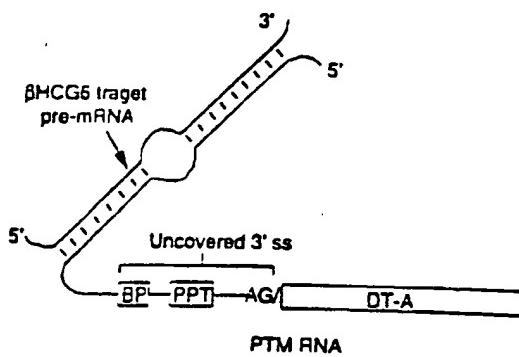


Figure 4 A-B

(C)

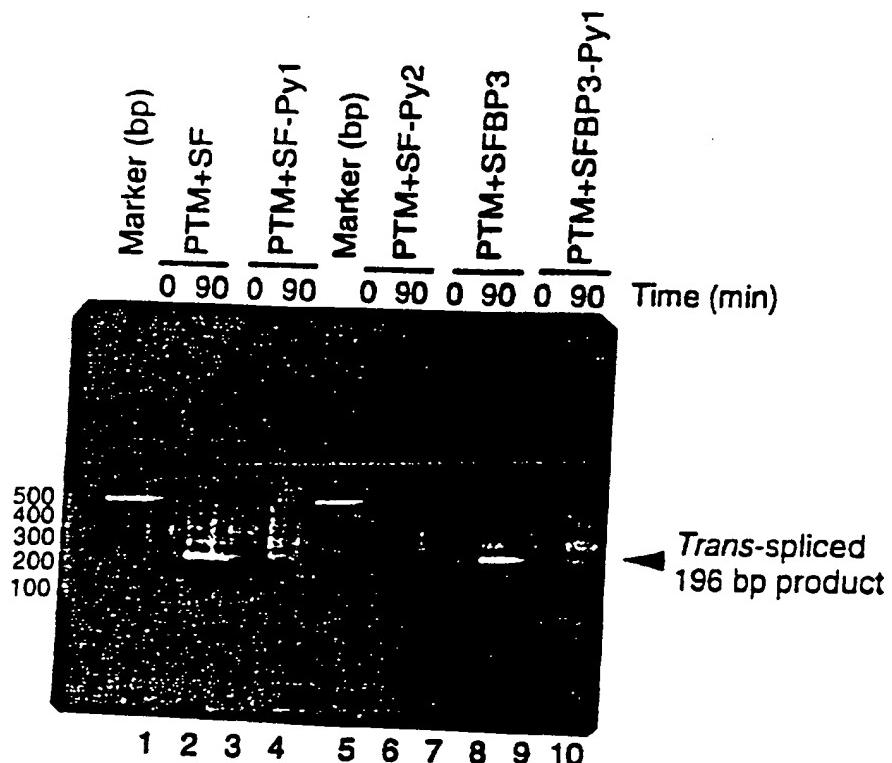


Figure 4c

51504B-A
(Sheet 7 of 8)

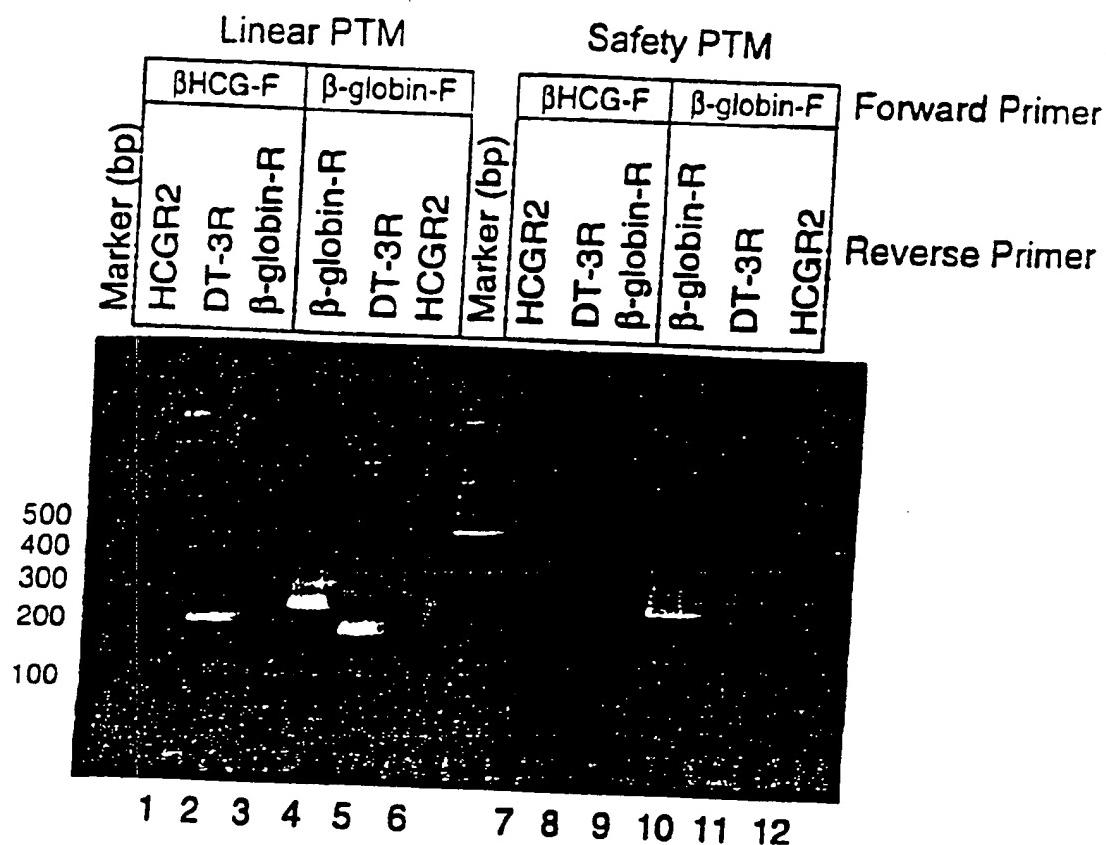


Figure 5

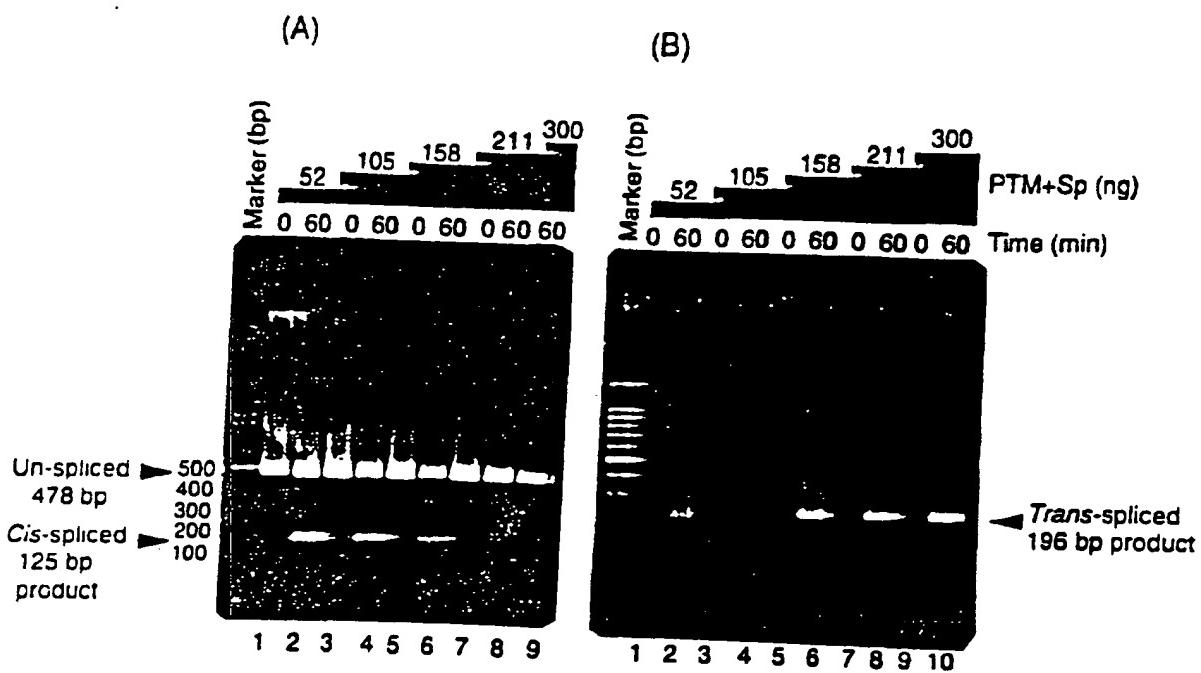
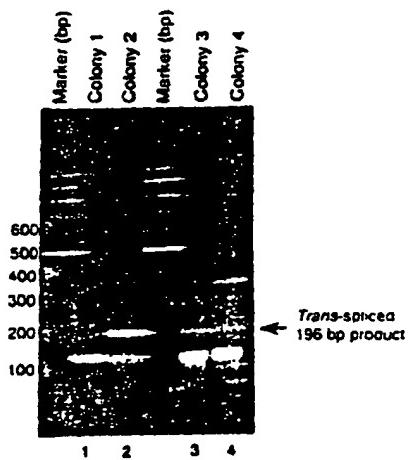


Figure 6

51504D-M1
(Sheet 9 Of 58)

Figure 7

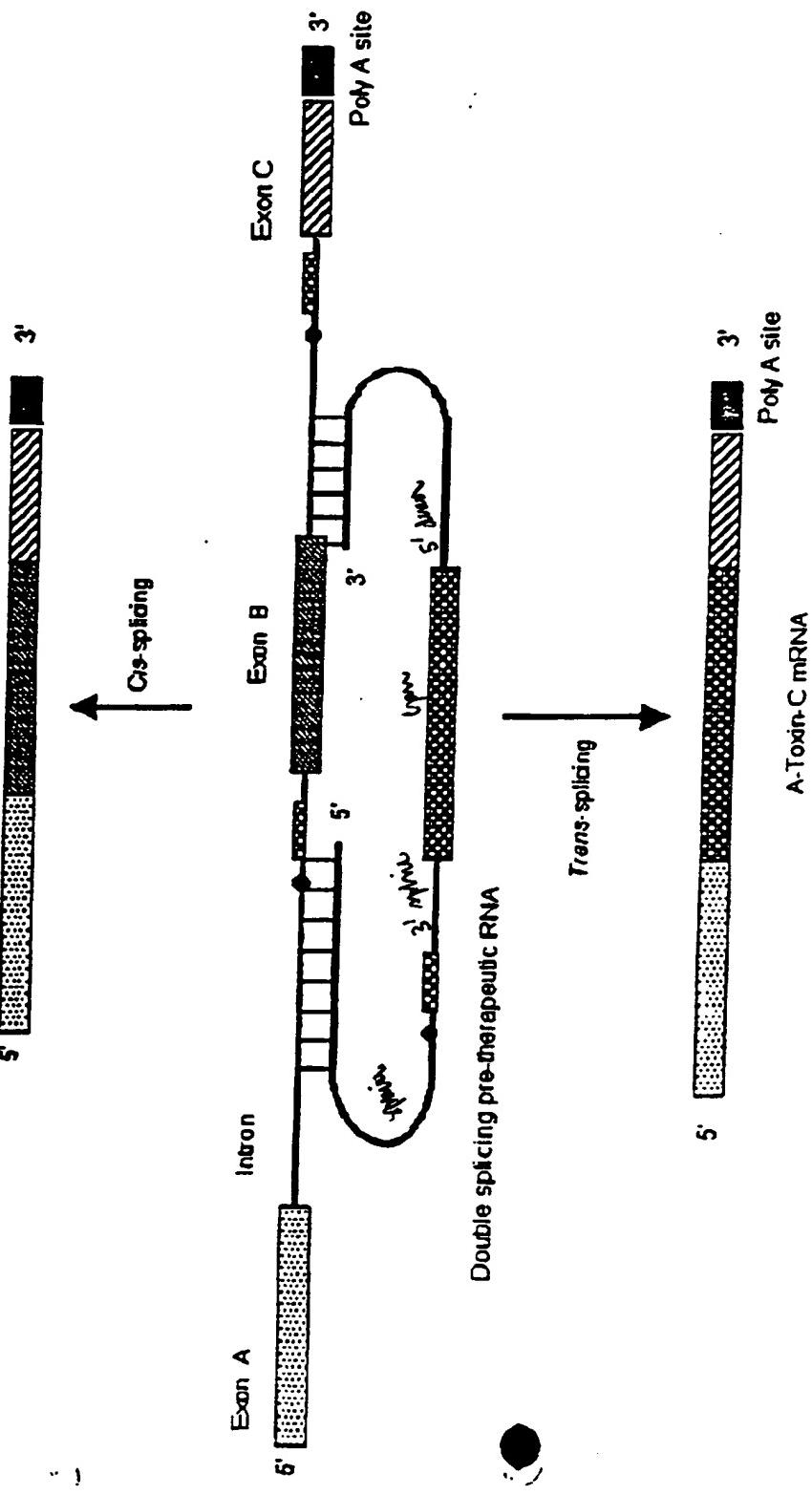
(A)



(B)

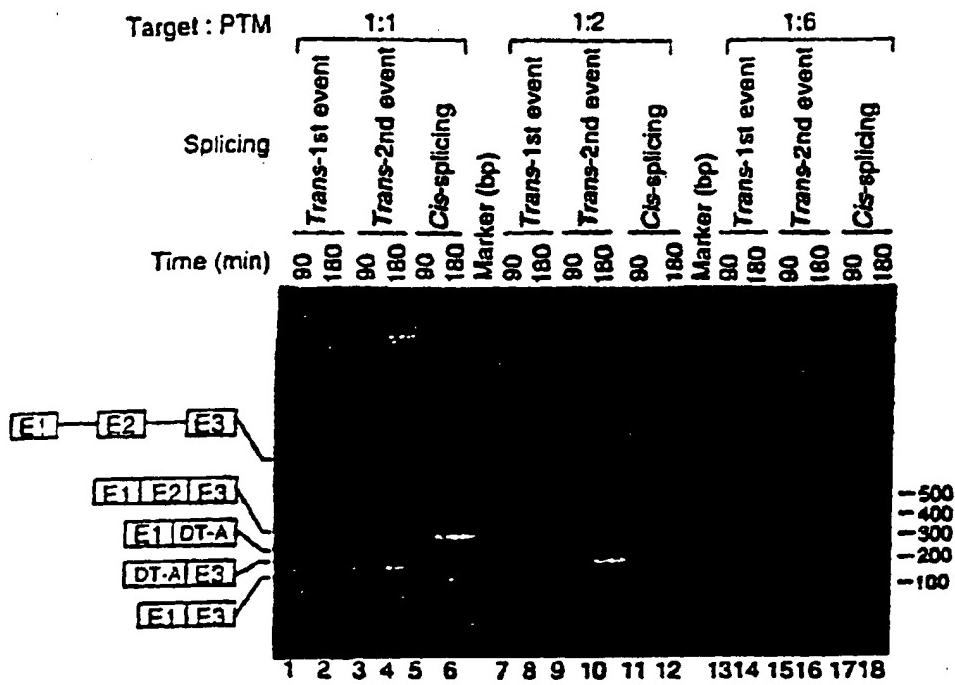
Exon 1 of β HCG6 ↓
5'-CAGGGGACGCACCAAGGATGGAGATGTTCCAG-GGCCGCTGATGATGTTGTT
↓ 1st coding nucleotide of DT-A
GATTCTTCTTAATCTTTGTGATGGAAAACCTTCTTCTGTACCAACGGGACTA
AACCTGGTTATGTAGATTCCATTCAAAAA - 3'

Double Splicing Pre-therapeutic RNA

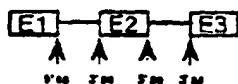


Selective Trans-splicing of a Double Splicing PTM

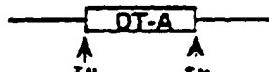
(3' ss of PTM to 5' ss target and, 5' ss of PTM to 3' ss of target)



BHCG Target



Double splicing PTM



Cis-spliced products

E1 | E2 | E3 = Normal cis-splicing (277bp)

E1 | E3 = Exon skipping (110bp)

Trans-spliced products

E1 | DT-A = 1st event, 196bp. Trans-splicing between 5' ss of target & 3' ss of PTM.

DT-A | E3 = 2nd event, 161bp. Trans-splicing between 3' ss of target & 5' ss of PTM.

Figure 8B

31304B-A

(Sheet 11 Of 58)

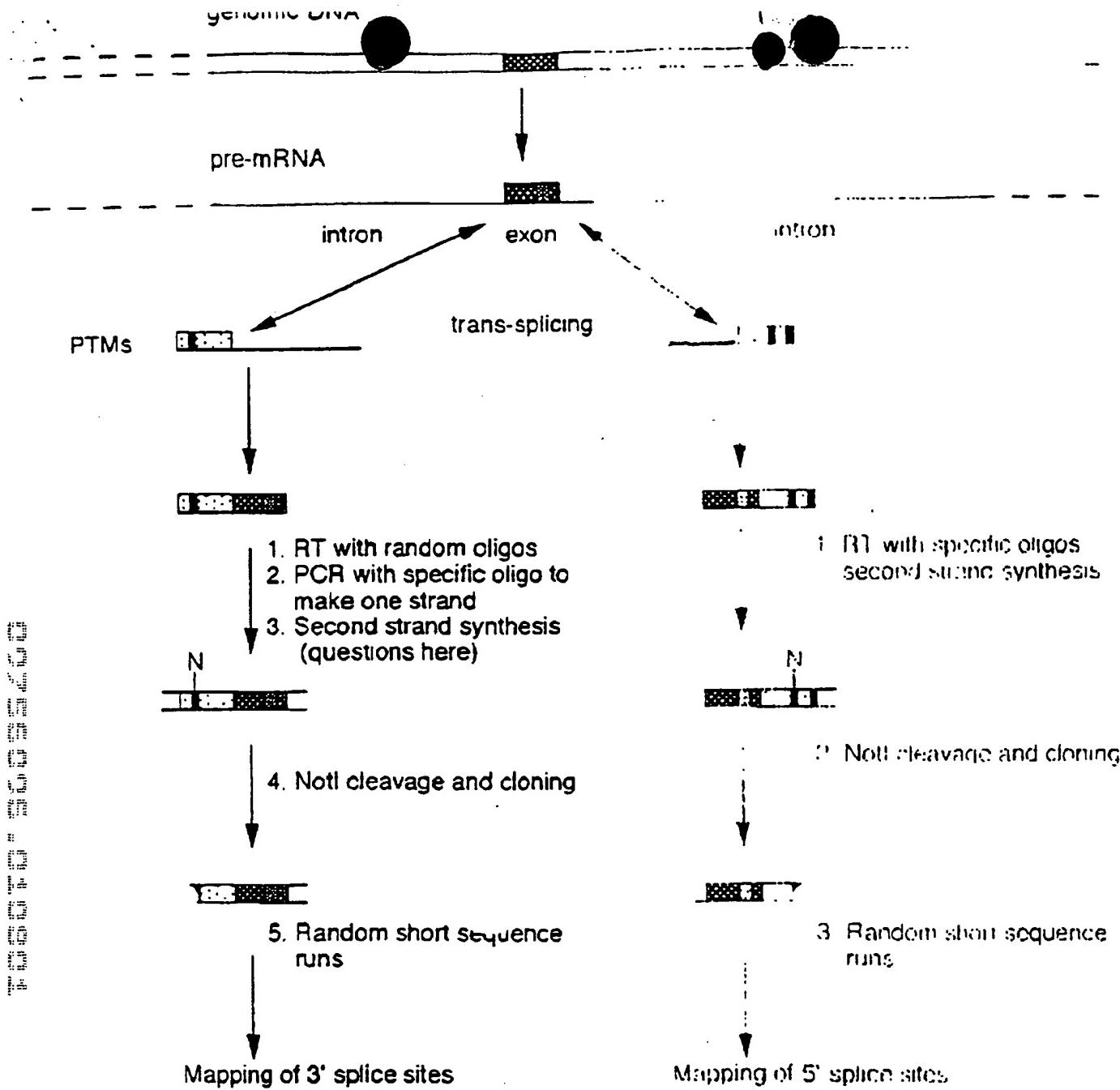


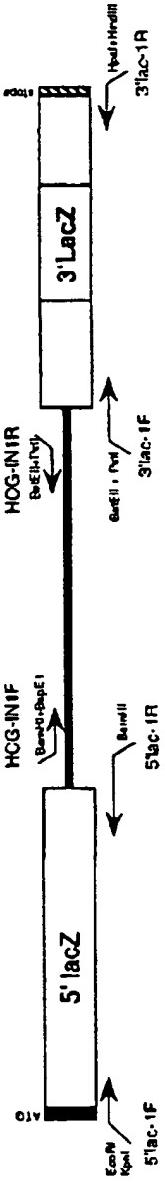
FIGURE 9

31304B-A
(Sheet 12 Of 58)

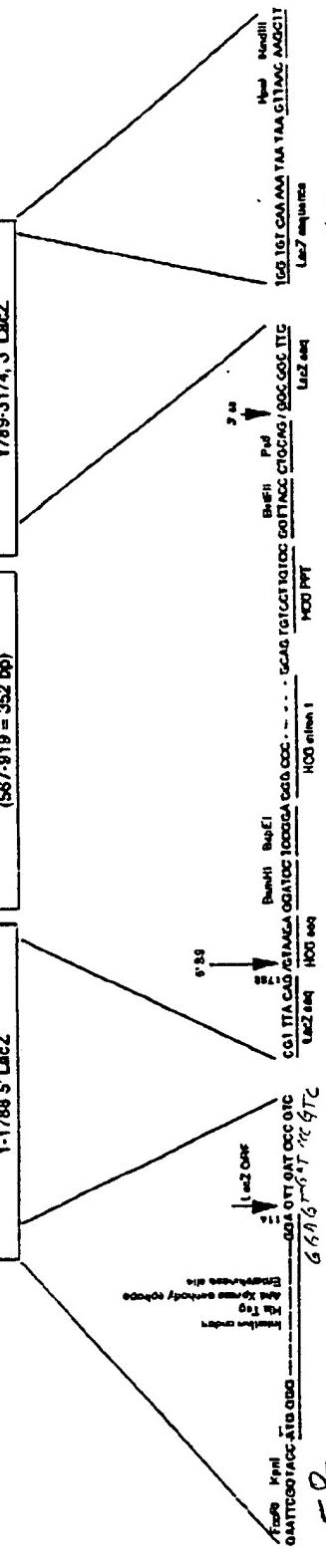
Knock Out LacZ Model Constructs

$\rho_{c3\cdot1} Lac\cdotT1$

Target 1:



B/HCG6 Intron 1
(587-919 = 352 bp)



58

62

59

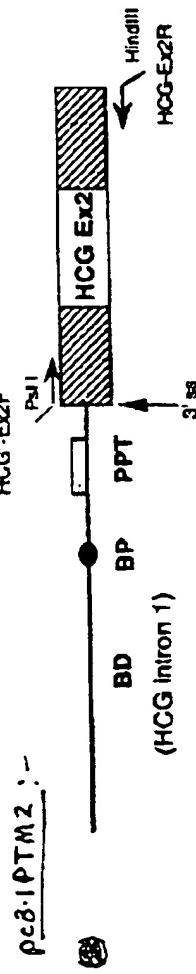
60

68

59

PTMs

$\rho_{c3\cdot1} PTM2$:-

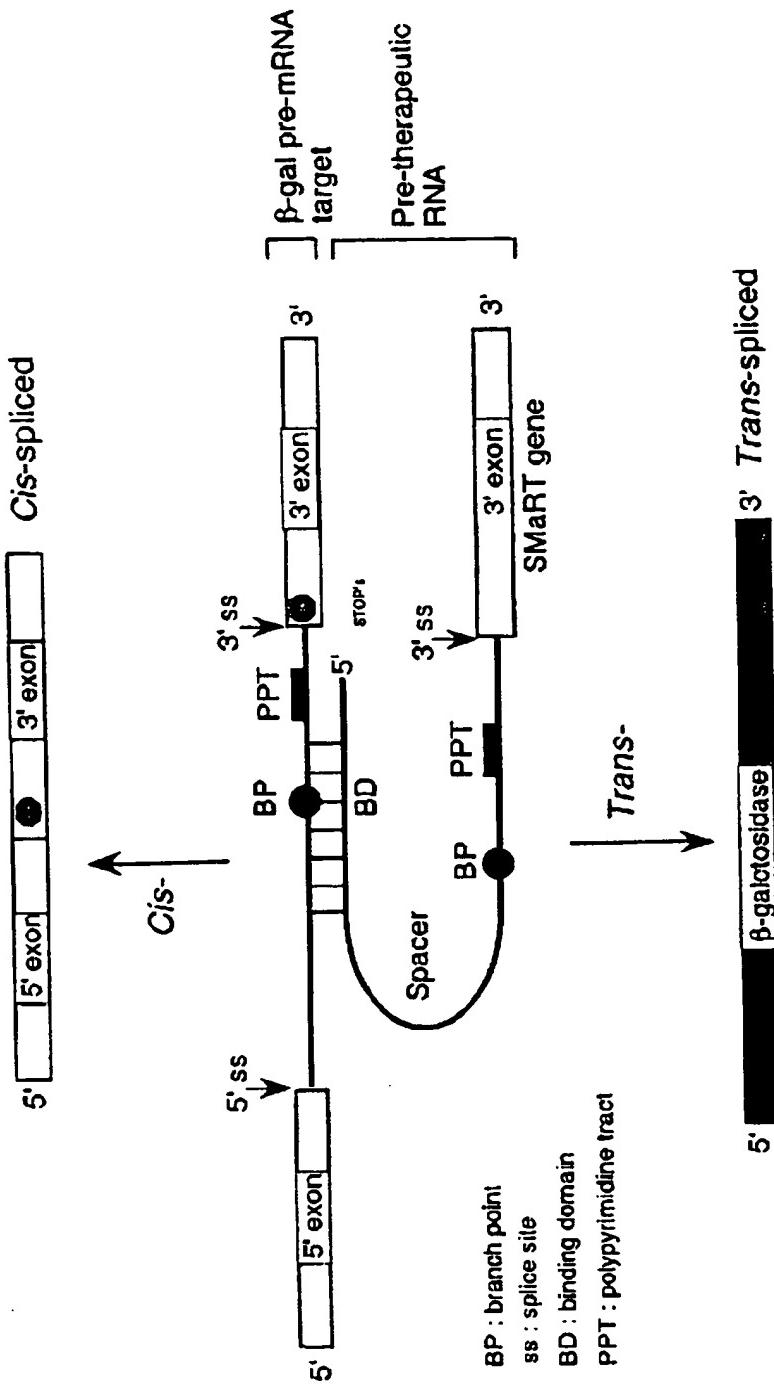


31304 B-A
(sheet 13 of 58)
FIG. 10 A

Restoration of β -Gal activity by SMArt (Spliceosome Mediated RNA Trans-splicing)

31304-B-A
for 14 of 14

Figure 1D B



31304 B-A
(Sheet 15 of 58)

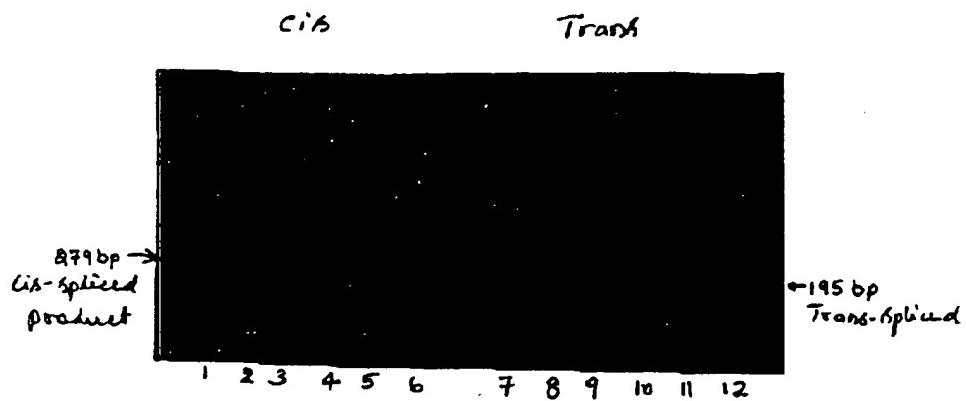


FIGURE 11A

31304 B-A
(Shut 16 of 58)

Figure 11B

PILOT D-11
(Sheet 17 of 58)

RECORDED BY R. L. HARRIS ON 10-11-68

FIGURE 11C

Nucleotide Sequence Demonstrating that Trans-splicing is Accurate

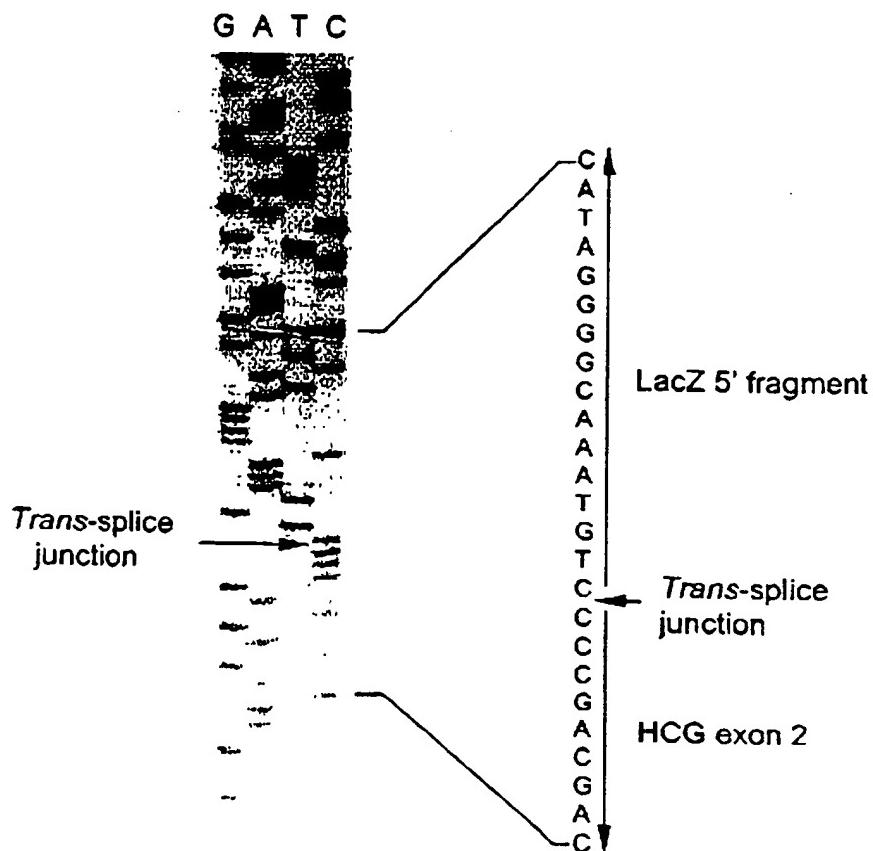


FIGURE 12 A

31304-B-A
(Sheet 18 of 58)

(1). Nucleotide sequences of the cis-spliced product (285 bp) :

BioLac-TR1

GGCTTTCGCTACCTGGAGAGACGCGCCCGCTGATCCTTGCAATAACGCCACGCGATGGGTAAACAGTCTTG

3

Splice junction

GGCGTTTCGCTAAATACCTGGCAGGCCTTCGTCAAGTATCCCCGTTACAG/GGCGGCTTCGTCAATAATG

GGACTGGGTGATCAGTCGCTGATTAAATATGATGAAAAACGCCAACCGTGGTCGGCTTACGGCCGGTGA

TGGCGATACGCCAACGATCGCCAGTTCTGTATGAACCGTCTGGCTTPGGCACCGCACGCCGCATCCAG Lac-TR2

(2) Nucleotide sequences of the trans-spliced product (195 bp)



BioLac-TR1

GGCTTTGCTACCTGGAGAGACGCCCGCTGATCCTTGCATAACGCCACGCGATGGTAAACAGTCTTGG

Splice junction

CGGTTTCGCTAAATACTGGCAGGCCTTCGTCACTATCCCCGTTACAG/GGGCTGCTGCTTCTGCTGCT

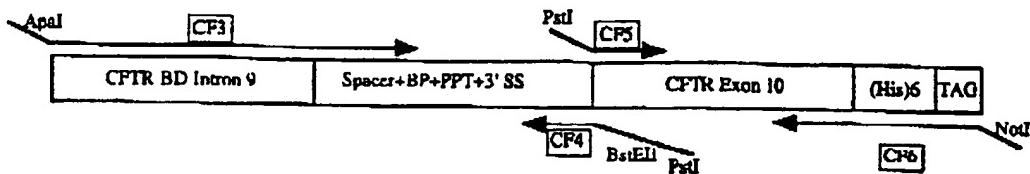
HCGR2

GAGCATGGGCGGGACATGGGCATCCAAGGAGCCACTTCGGCCACGGTGCCG

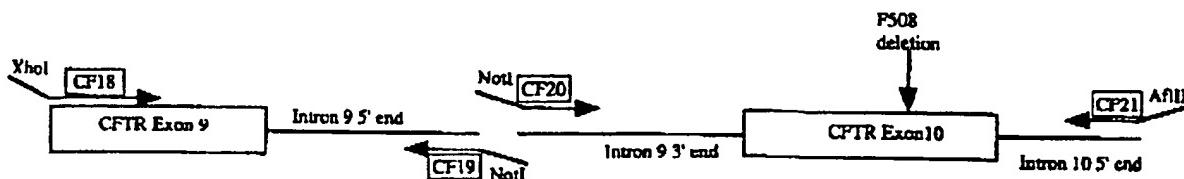
Figure 12 B

31304-B-A
(Sheet 19 of 58)

CFTR Pre-therapeutic molecule (PTM or "bullet")



CFTR mini-gene target - Construction



TRANS- SPLICING Repair

Binding
of
PTM to TARGET

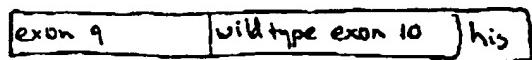
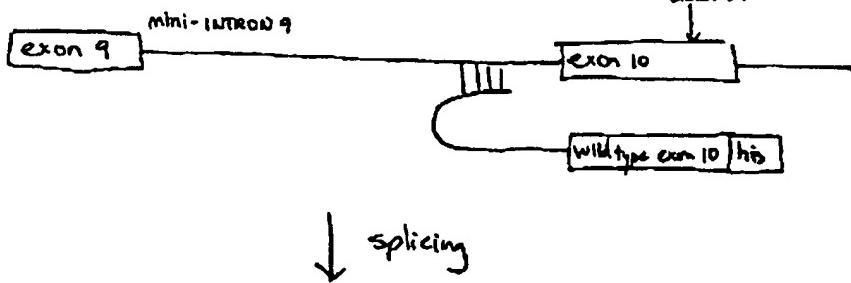
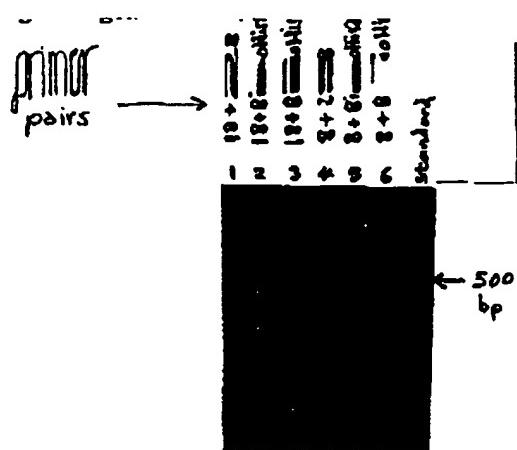


Figure 13

31304-B-A
(Sheet 20 of 58)

Figure 14

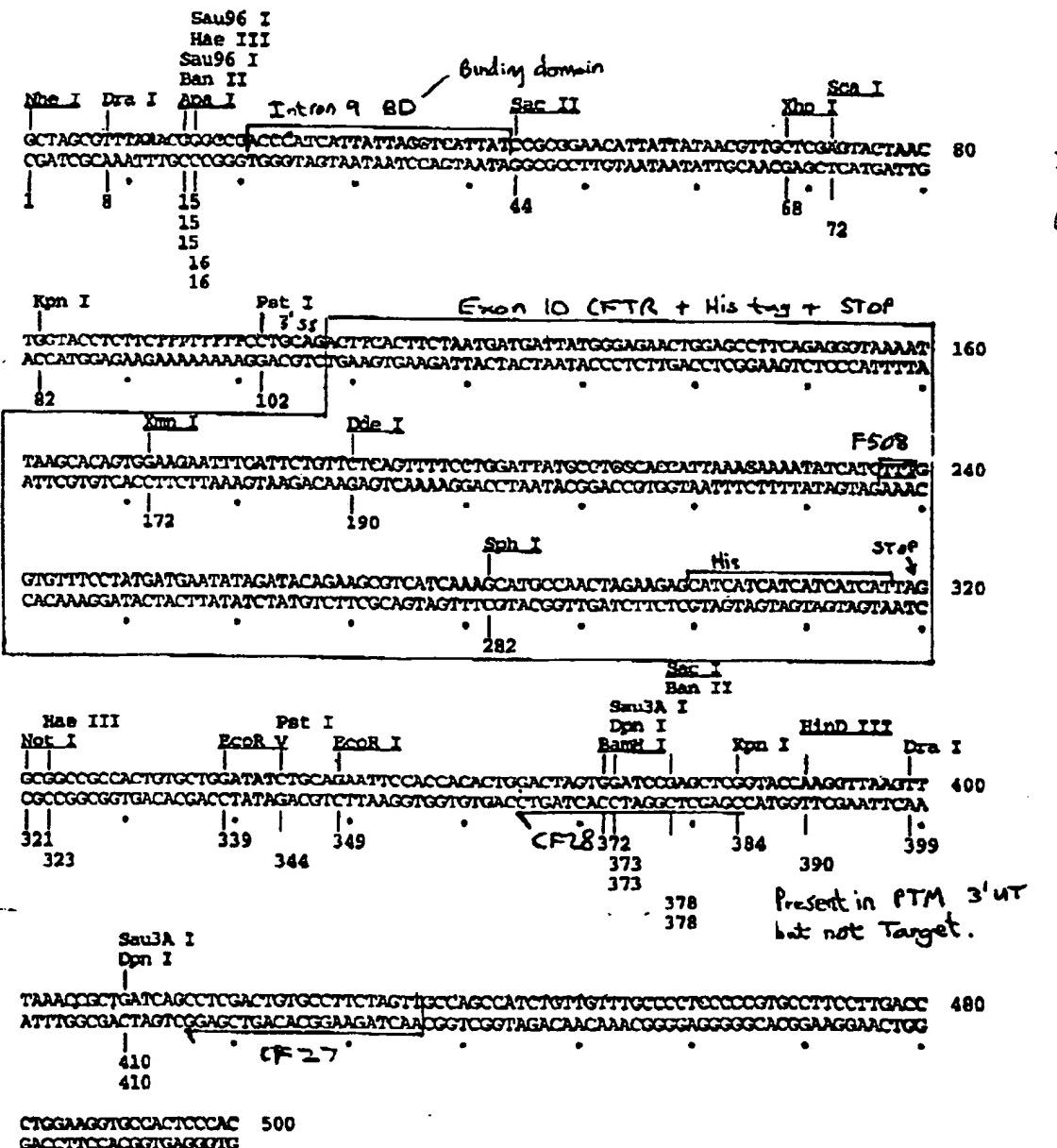


31304 B-A
(Shut 21 of 58)

FIGURE 15

DNA sequence 500 b.p. GCTAGCGTTAA ... TGCCACTCCAC linear /

Positions of Restriction Endonucleases sites (unique sites underlined)



Restriction Endonucleases site usage

Acc I	-	EcoR I	1	Nde I	-	Sau96 I	2
Apa I	1	EcoR V	1	Nhe I	1	Sca I	1
Xba I	-	Hae III	-	Not I	1	Sma I	-
Xba II	-	Hae III	2	PflM I	-	Sph I	1
BamH I	1	Hinc II	-	Pst I	2	Spl I	-
Ban II	2	Hind III	1	Pvu I	-	Ssp I	-
Bbv I	-	Hinf I	-	Pvu II	-	Stu I	-

31304-A-B
(Shut 27 of 58)

EXPERIMENT 12

Repair of an exogenously supplied CFTR target molecule carrying an F508 deletion in exon 10.

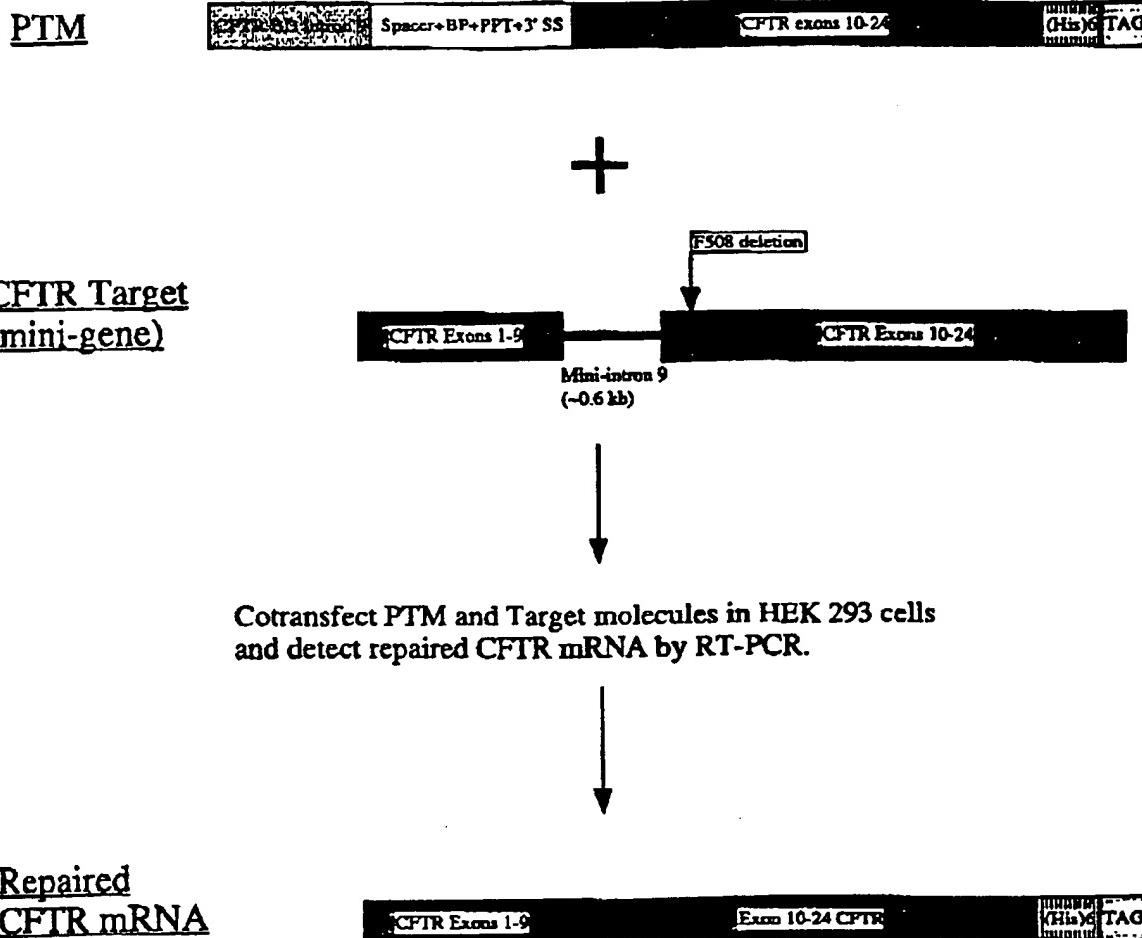


Figure 1b
31304-A-B
sheet 23 of 58

EXPERIMENT 3

Repair of endogenous CFTR
transcripts by exon 10 invasion
using a double splicing PTM

Double Splicing
PTM

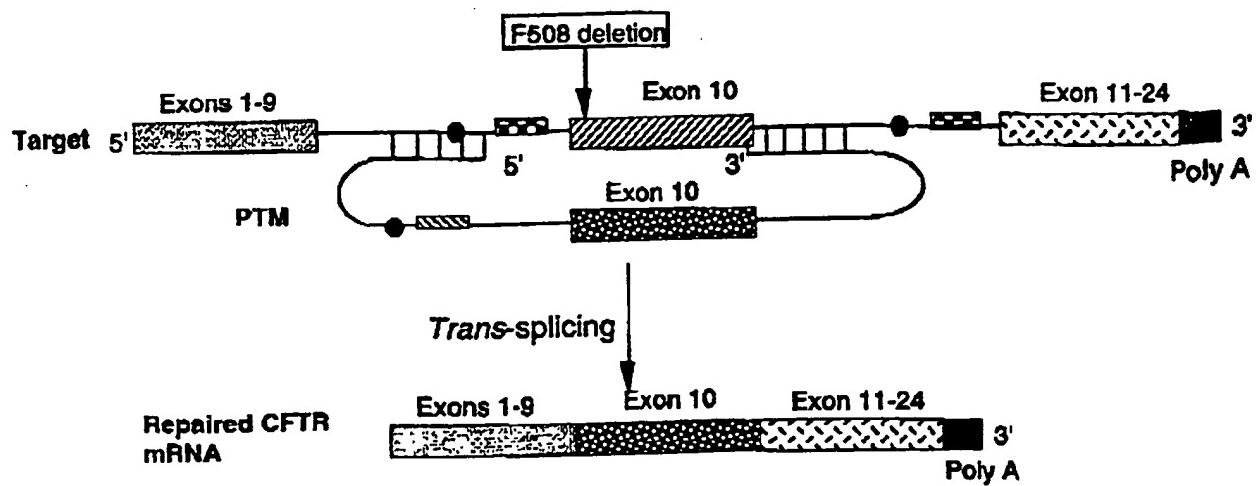


Figure 17

31304 B-A

Sheet 24 of 58

Double Trans-splicing Specific Target

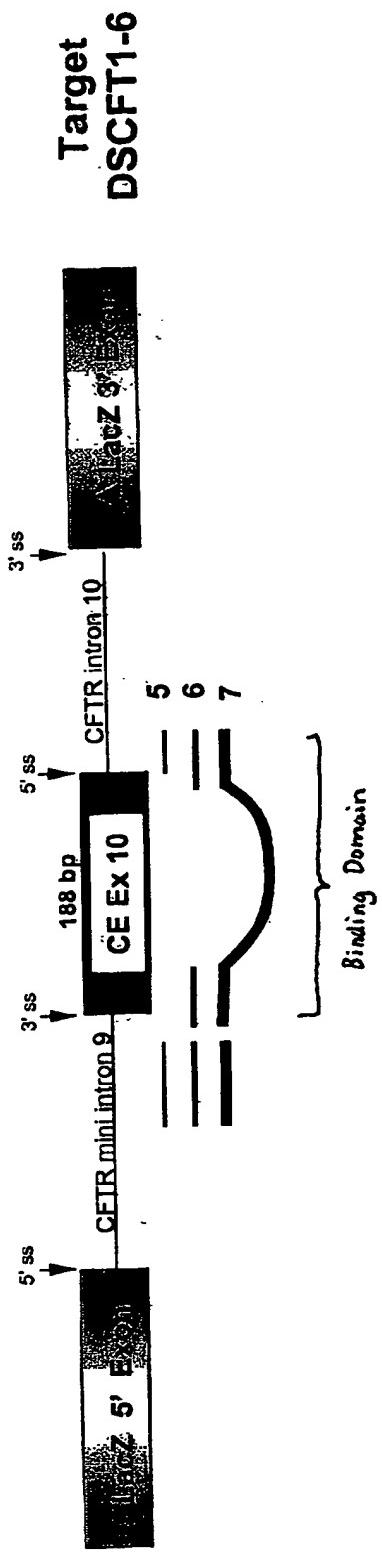


Figure 18

about 25 to 58

Double Trans-Splicing PTMs

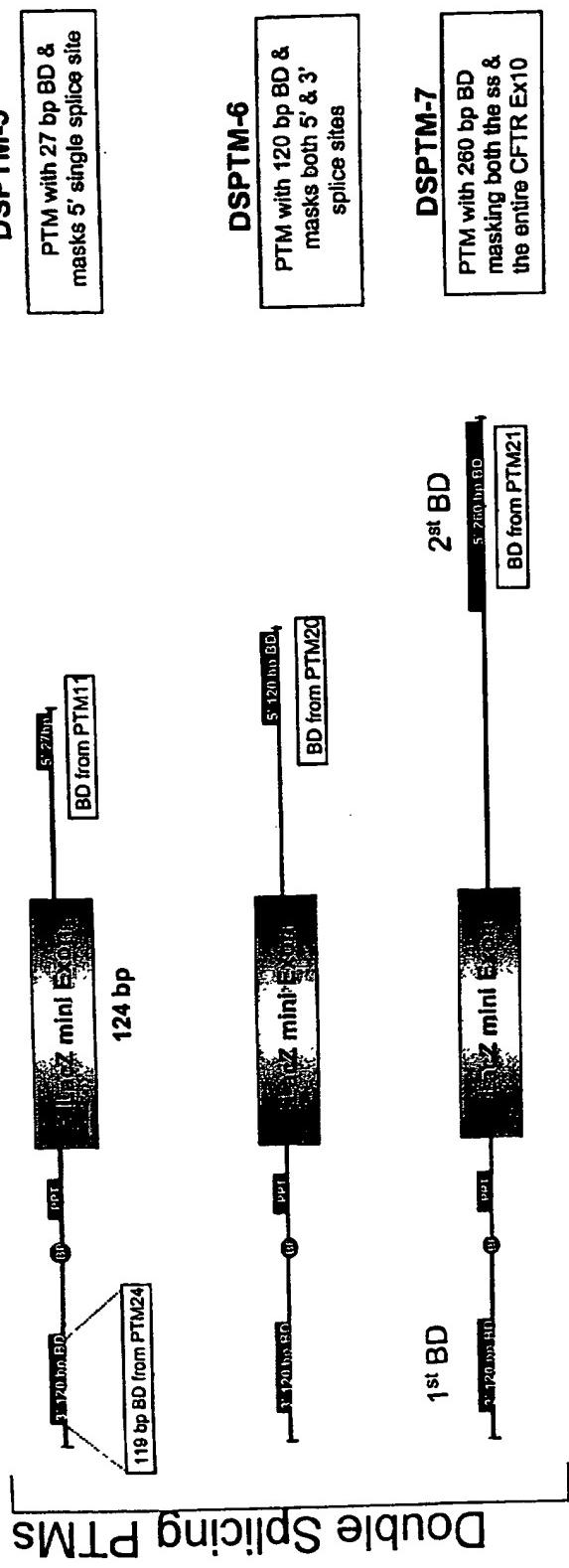
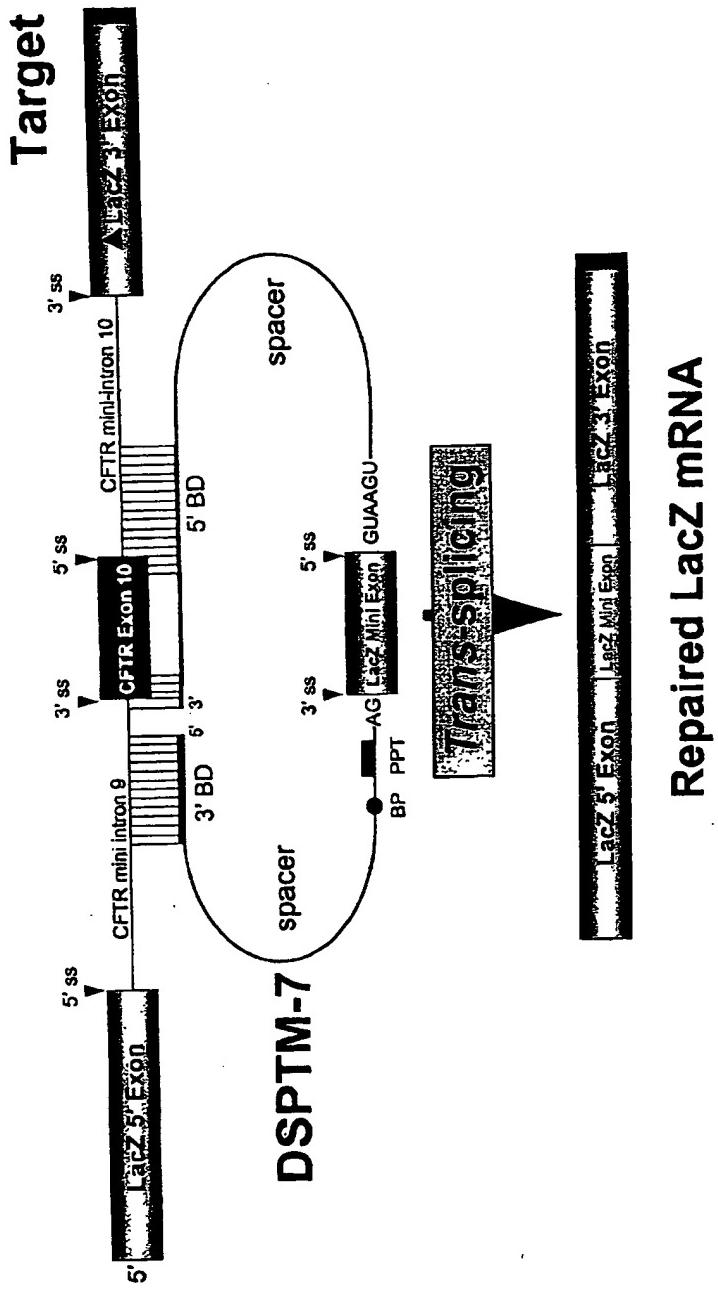


Figure 19

about 26 of 58

Double Trans-splicing β -Gal Model

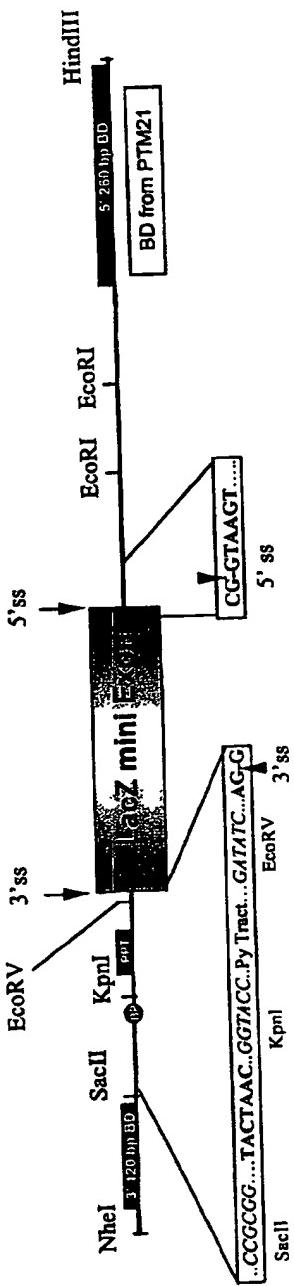


Repaired LacZ mRNA

Figure 20

85 to t2 myr

Important Structural Elements of DSPTM-7: (Double splicing PTM with all the necessary splice elements i.e. has both 3' and 5' functional splice sites and the binding domains)



(1) 3' BD (120 BP) : GATTCACTTGCCTCCAAATTATCATCCTAAGCAGAAGTGTATATTCTTATTGTAAAGATTCTATTAACTCATTGATTCAAAATTTAAAATACTTCCCTGTTCATACTCTGCTATGCAC

(2) Spacer sequences (24 bp): AACATTATTATAACGTTGCTCGAA

(3) Branch point, pyrimidine tract and acceptor splice site: TACTAACATTGGTACCTCTTTCTTTT GATATCCTGCAG CTGCAG GCGCG

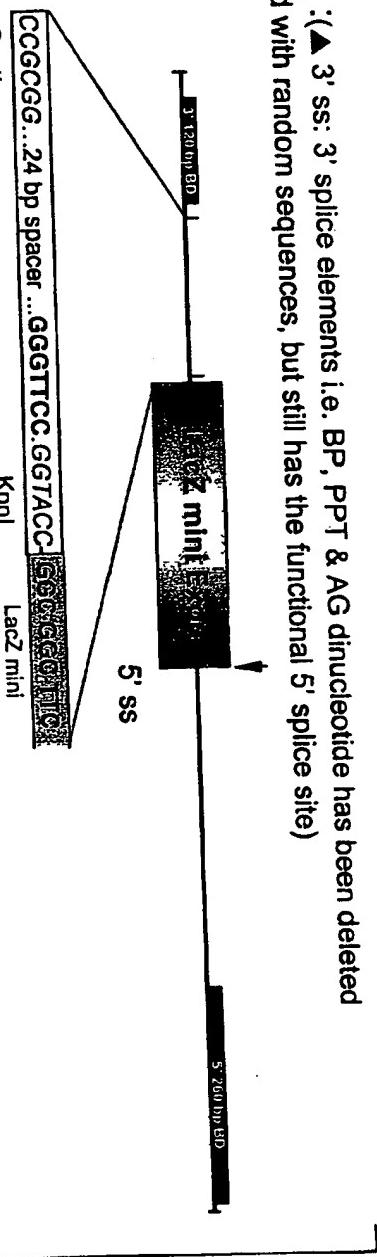
(4) 5' donor site and 2nd spacer sequence: TCAAG GTAAGT GTTATCACCGATATGTGTCTAACCTGATTCTGGCCCTTGATAACGCTTAAGATCCACCGG

(5) 5' BD (260 BP) : TCAAAAAGTTTCACTAAATTCTTACCTCTTCTGACGCTTCTGTATCTATATTCTCATTCATTGGAAACACCAATGATTTCCTGGCTGGCATATACTGGAAAACCTGATAACACAATGAAATTCTTCCACTGTGCTTAAAAAAACCCCTCTGAATTTCCTCCATTTCATCATTACAACACTGAAACTAAACCATTCATTAACTCA TTATCAAATCAGC

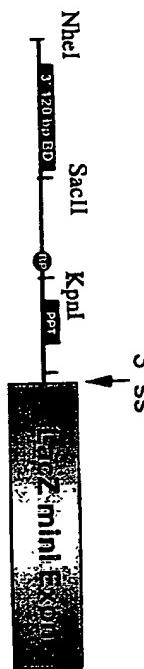
Figure 21

Sheet 29 of 58

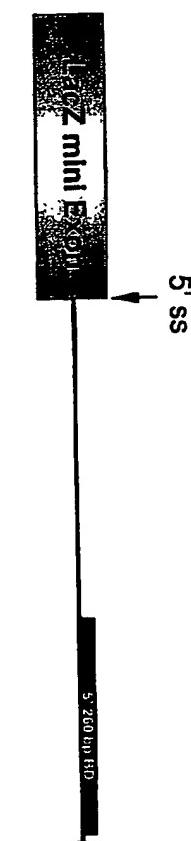
DSPTM8 (▲ 3' ss: 3' splice elements i.e. BP, PPT & AG dinucleotide has been deleted and replaced with random sequences, but still has the functional 5' splice site)



PTM29 (lacks 2nd BD and 5' ss)



PTM30 (lacks 1st BD and 3' ss)



Mutants

Figure 22

Accuracy of Double Trans-splicing Reaction

Street 30 of 58

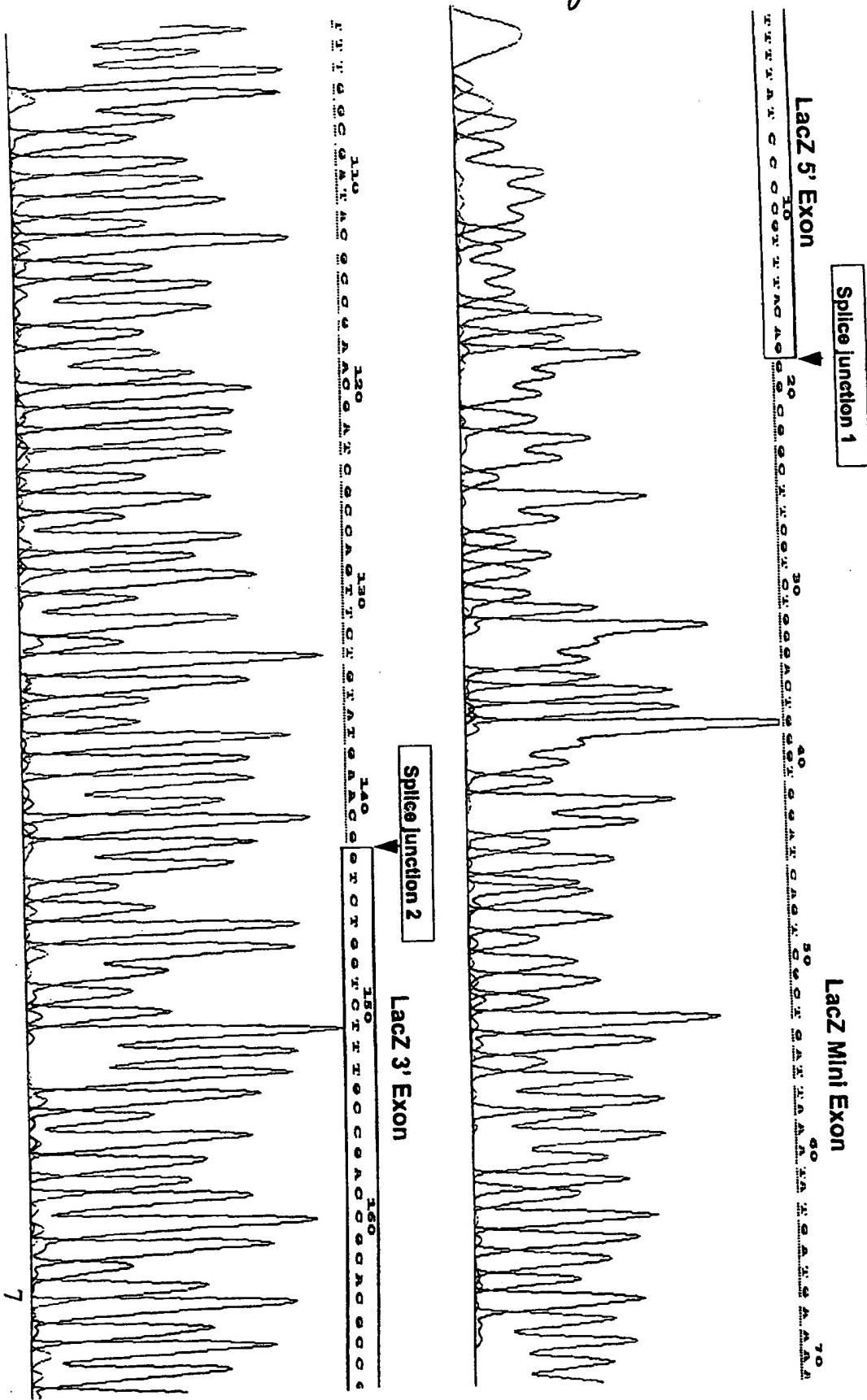
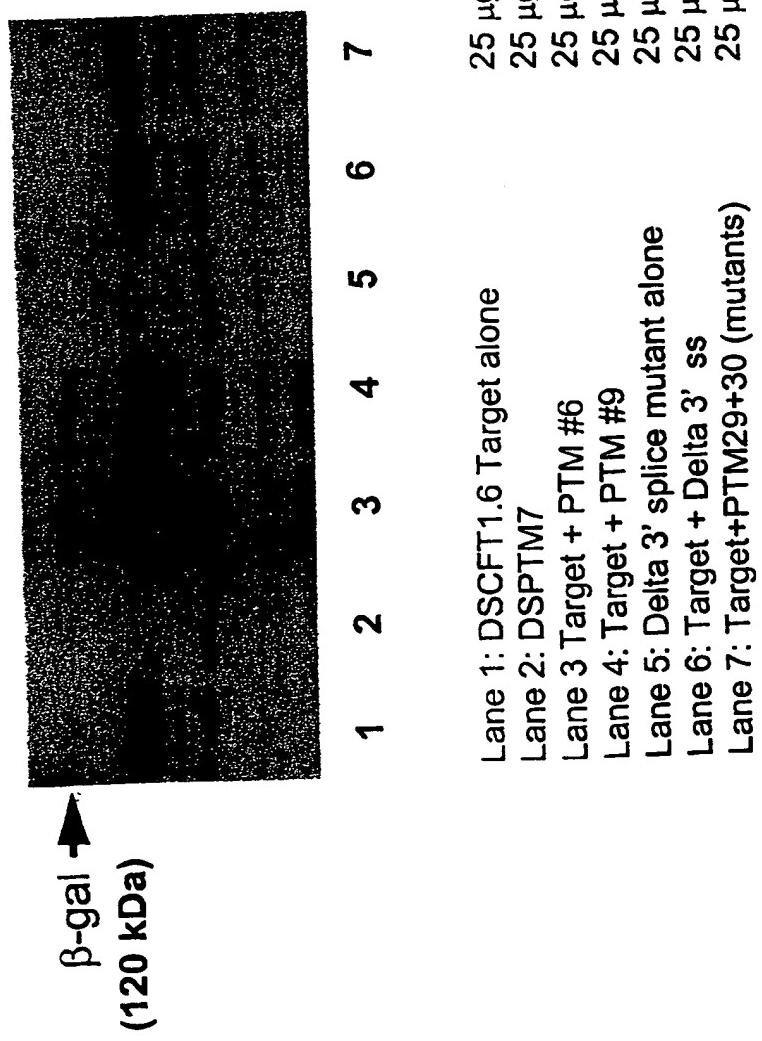


Figure 23

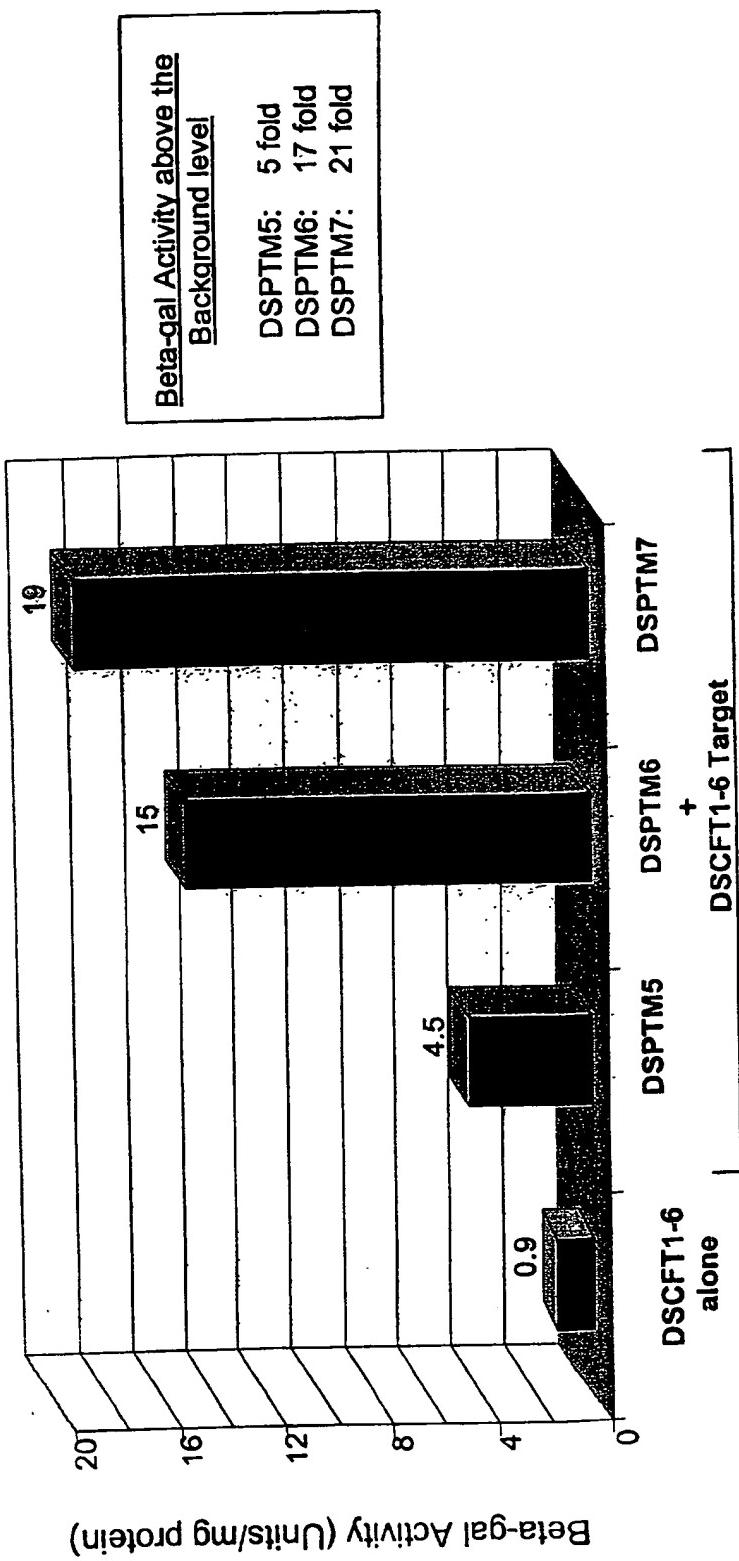
Double Trans-splicing Produces Full-length Protein



85 to 15 of 58

Figure 24

Restoration of β -Gal Function by Double Trans-splicing



11

Figure 25

Altmt 32 of 58

Restoration of β -gal activity is due to double RNA trans-splicing events

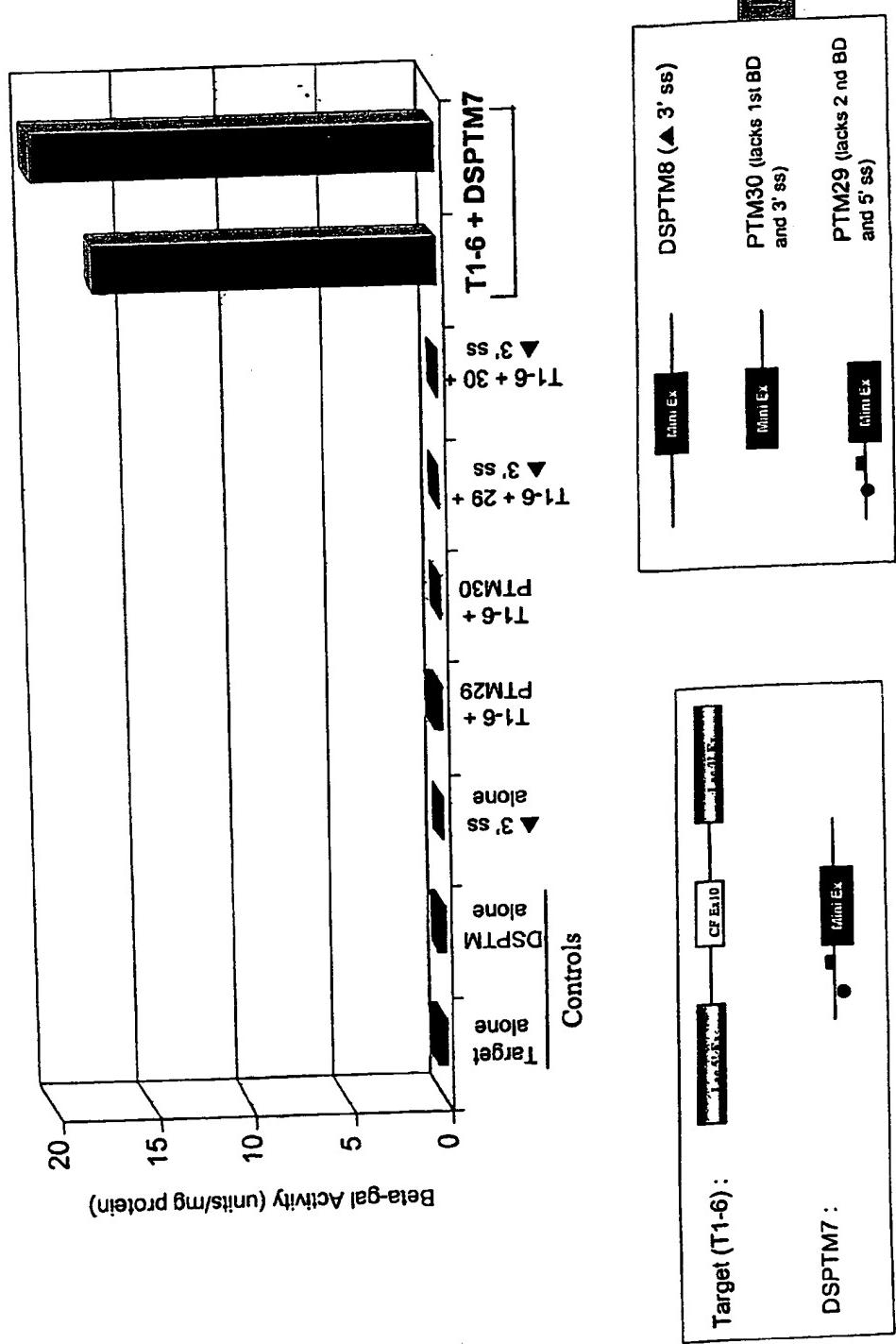


Figure 26

slide 33 of 58

Double Trans-splicing: Titration of Target & PTM

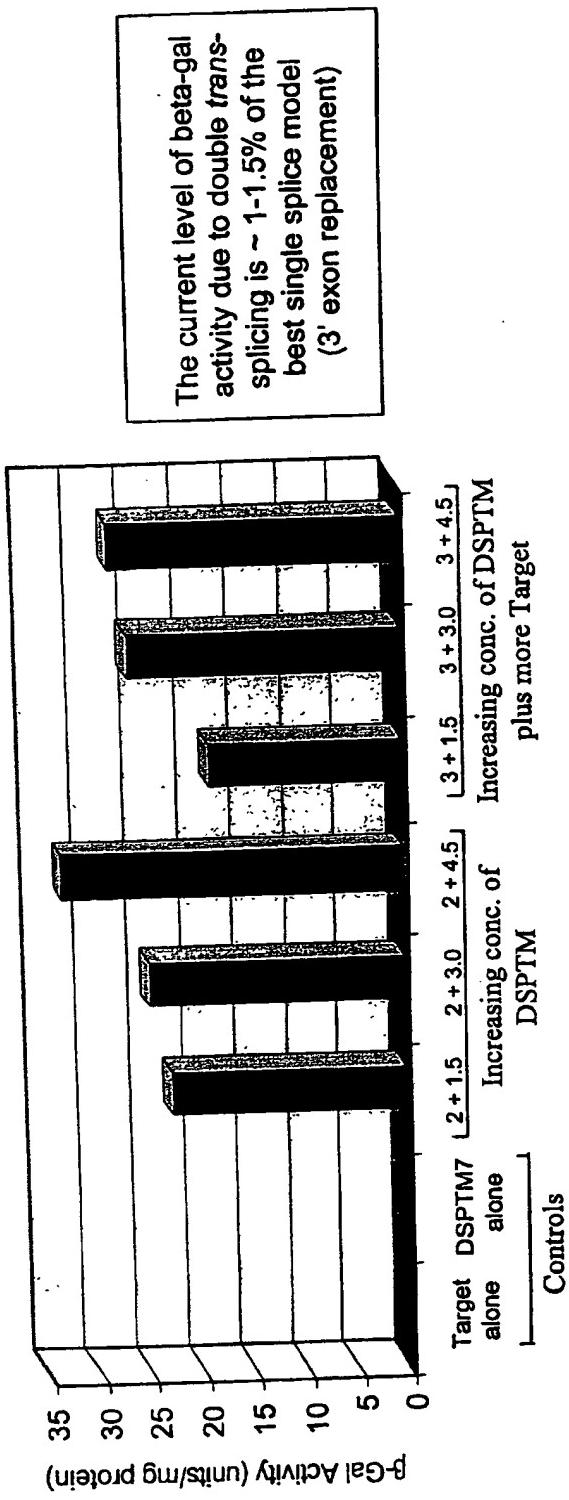
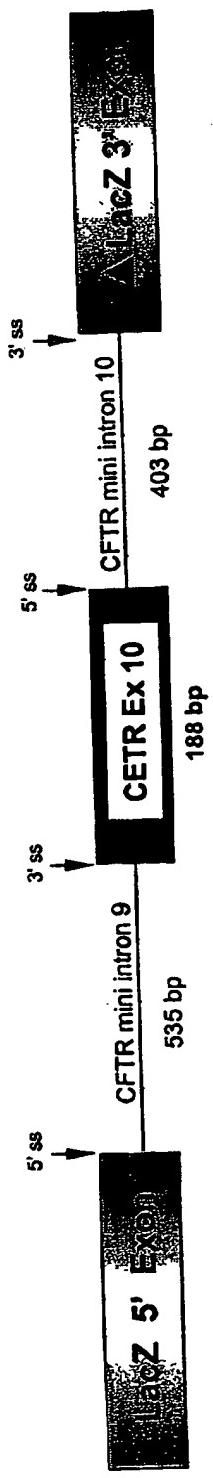


Figure 27

Chart 34 of 58

DSCFT1-6 (Specific Target):



DSHCGT1 (Non-specific Target):

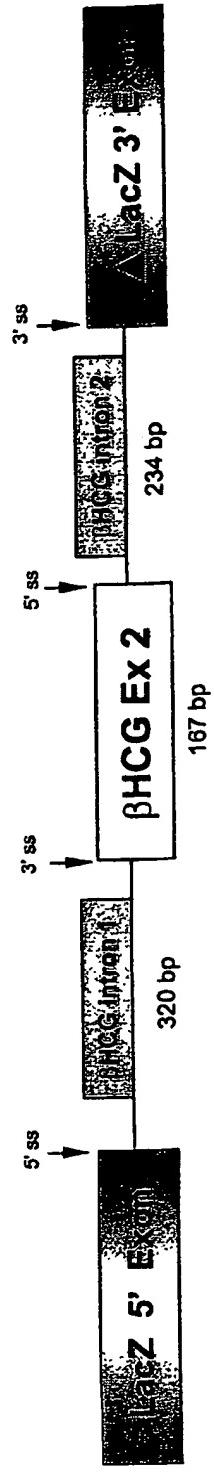


Figure 28

Chart 35 of 58

Specificity of double *trans*-splicing Reaction

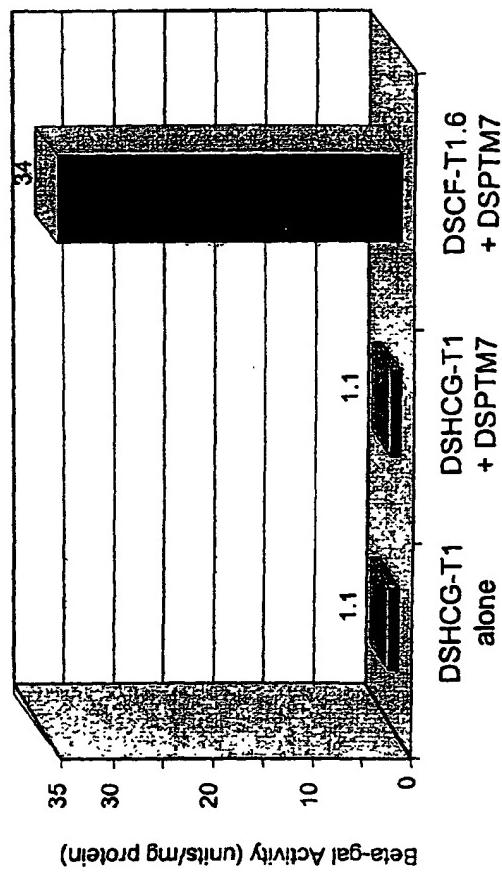
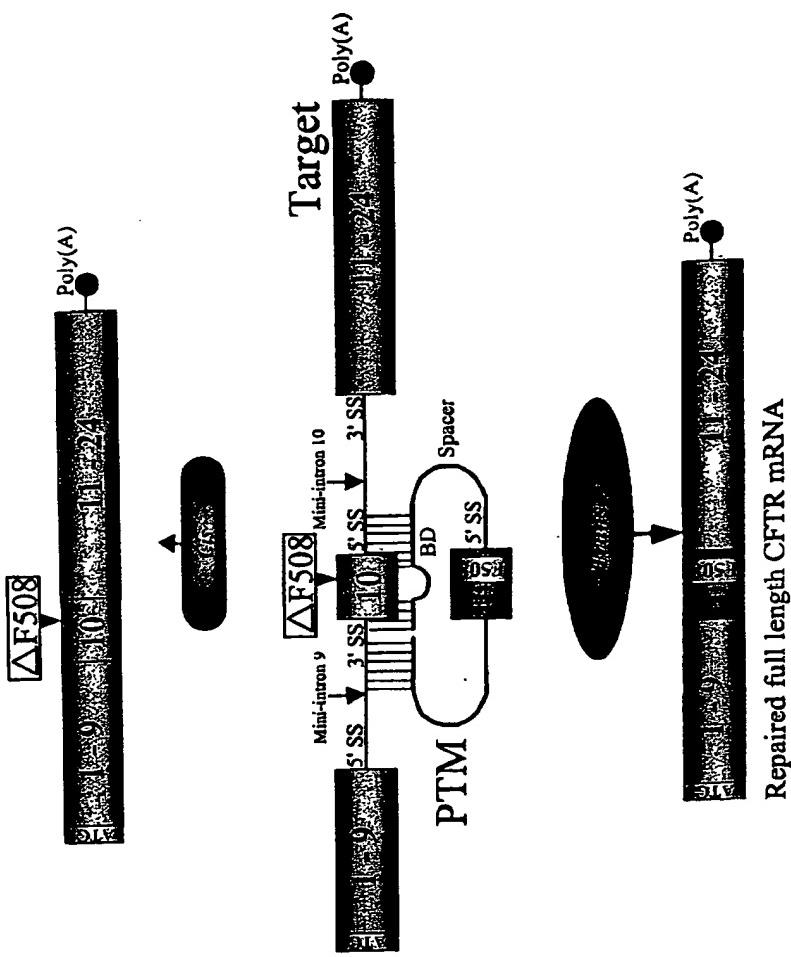


Figure 29

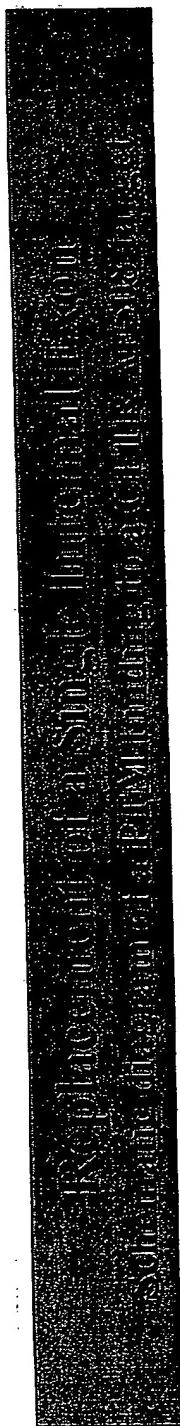
85 to 98%
achieved

NIRQNN
Figure 30



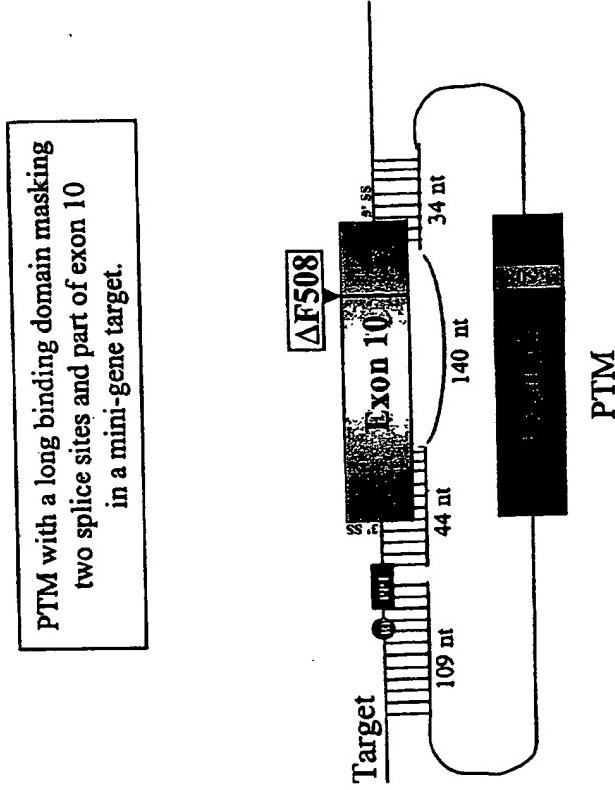
Repaired full length CFTR mRNA

about 38 nm



INTRON

Figure 3



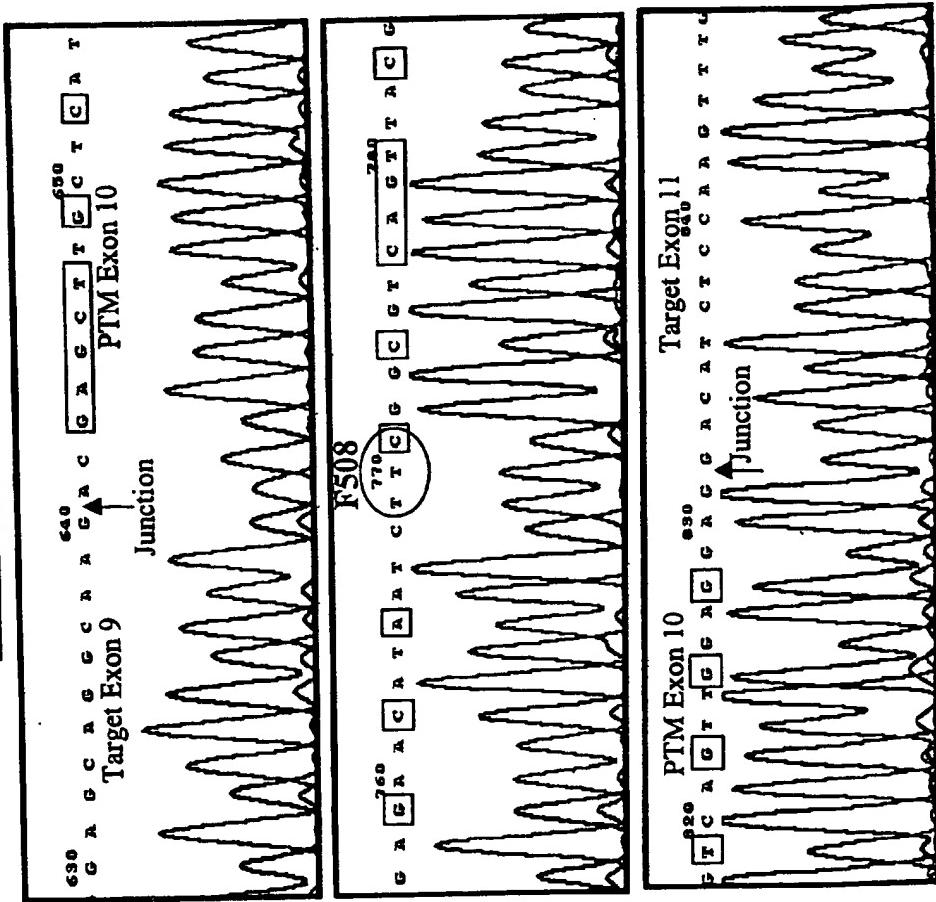
ACCAGCTTGCCTCATGATGATCATGCCCGAGT**TAGAACC**AAGTGAAGGGAAGATCAAACATTCCG
GCGC**ATCAGCTT**TGCAGCCAATTCAGTTGATCATGCCCGGTACCATCAAGGGAACATAAT
CTTGGCC**TCAGT**TACCGAAGTACCGAAGTACCGAAGTACCGAAGTACCGAAGTACCGAA

MCU in exon 10 of PTM

88 of 192 (46%) bases in PTM exon 10 are not complementary to its binding domain (bold and underlined).

85 to 86 myr

**Sequence of a double
trans-spliced product**



□ = MCU in
PTM exon 10

Figure 32

11

85 to 68 mdp

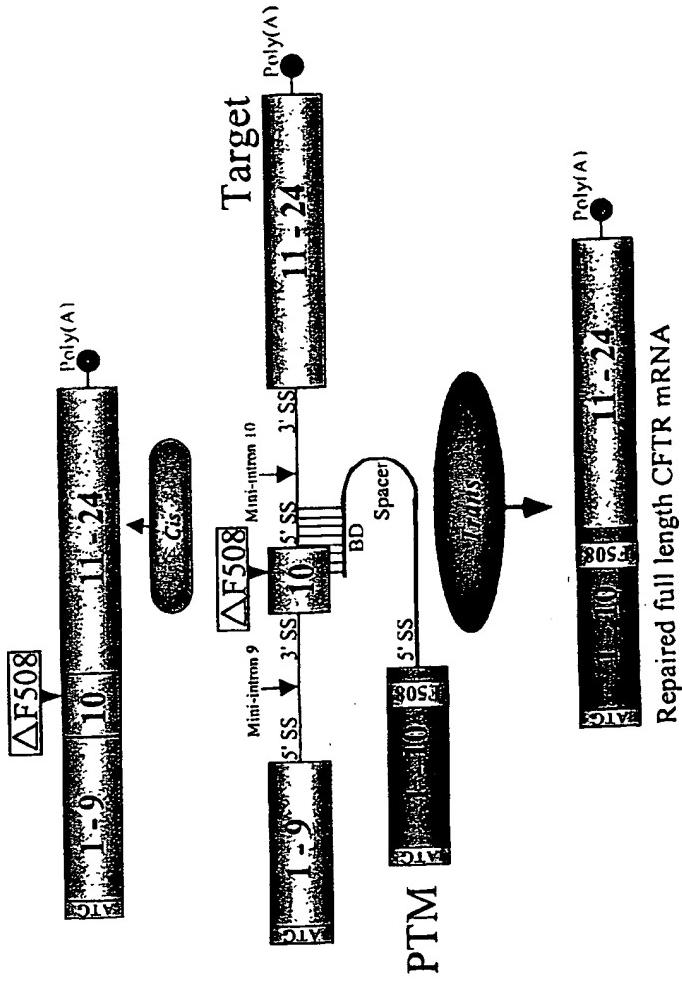
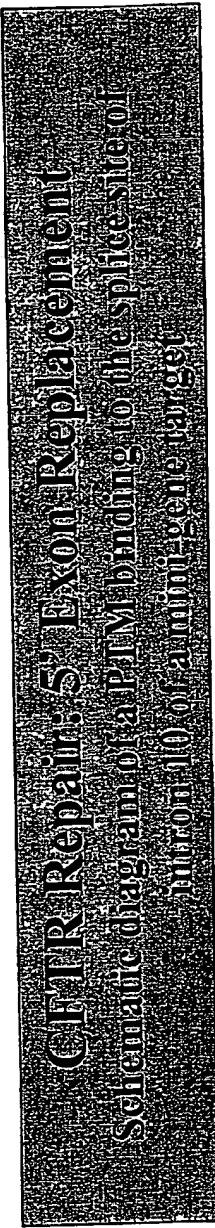


Figure 33

Target ss Exon 10 ss
 |
 4 nt

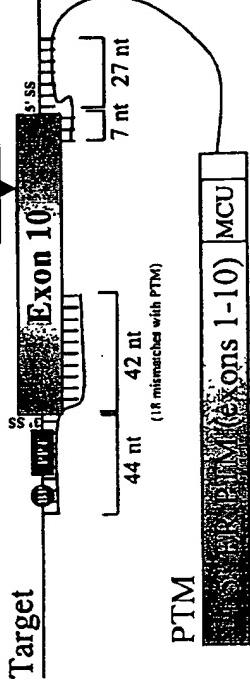
$\Delta F508$



A

PTM with a short binding domain masking a single splice site in a mini-gene target.

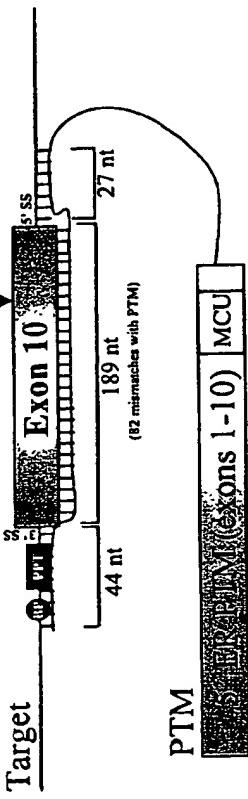
$\Delta F508$



B

PTM with a long binding domain masking two splice sites in a mini-gene target.

$\Delta F508$

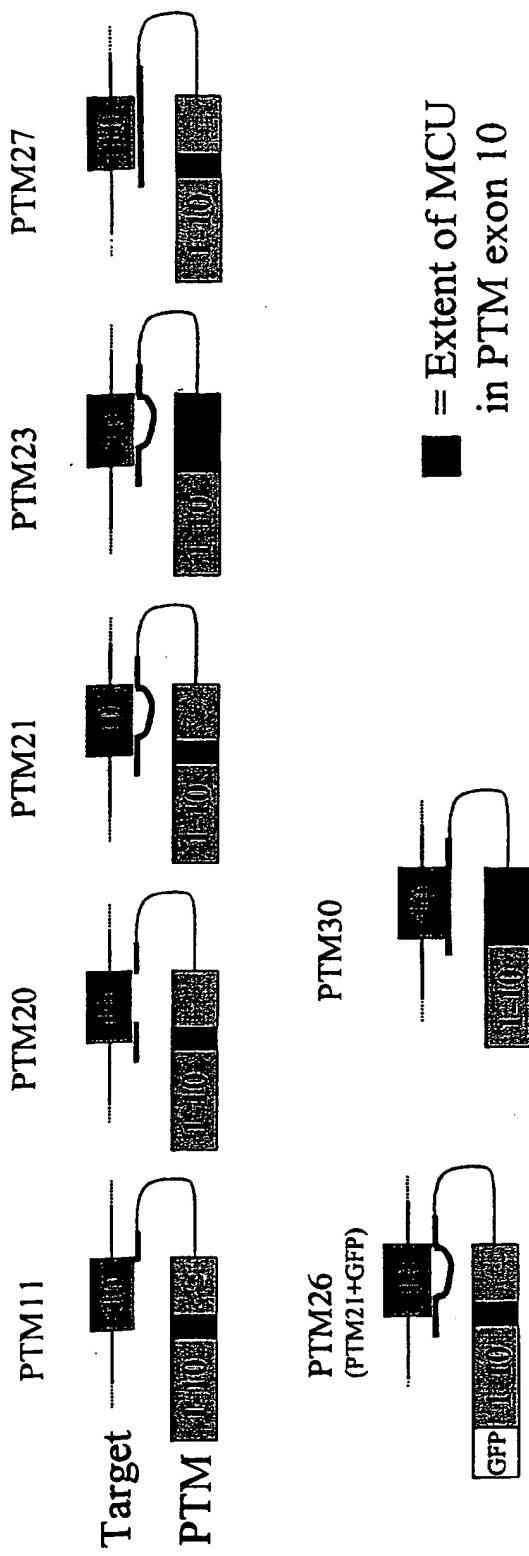


C

PTM with a long binding domain masking two splice sites and the whole of exon 10 in a mini-gene target.

Figure 34

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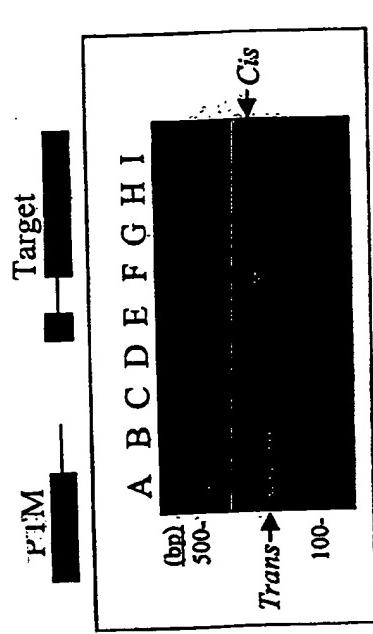


MCU in exon 10 of PTM
88 of 192 (46%) bases in PTM exon 10 are not complementary to its binding domain.

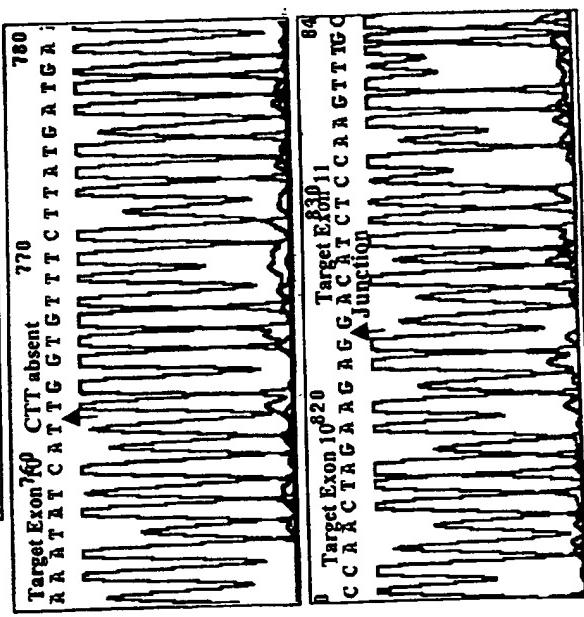
ACGAGCTTGCTCATGATGATCATGGGCCAGTTAGAACCAAGTGAAGGCCAAGATCAAACATTCCG
GCCGCATCAGCTTTGCAAGCCATTCAATTCAAGTTGGATCATGCCCGTACCATGAAAGGAACATAAT
CTTCGGCGTCAAGTTACGAGTAACGAGTACCGCTATCGCTCGGTGATTAAAGGCCTGTCAGTTGGAGGAG

Figure 35

INTRONIN



Cis-spliced product
[Primers CF1 + CF111]



Trans-spliced product
[Primers CF93 + CF111]

B.

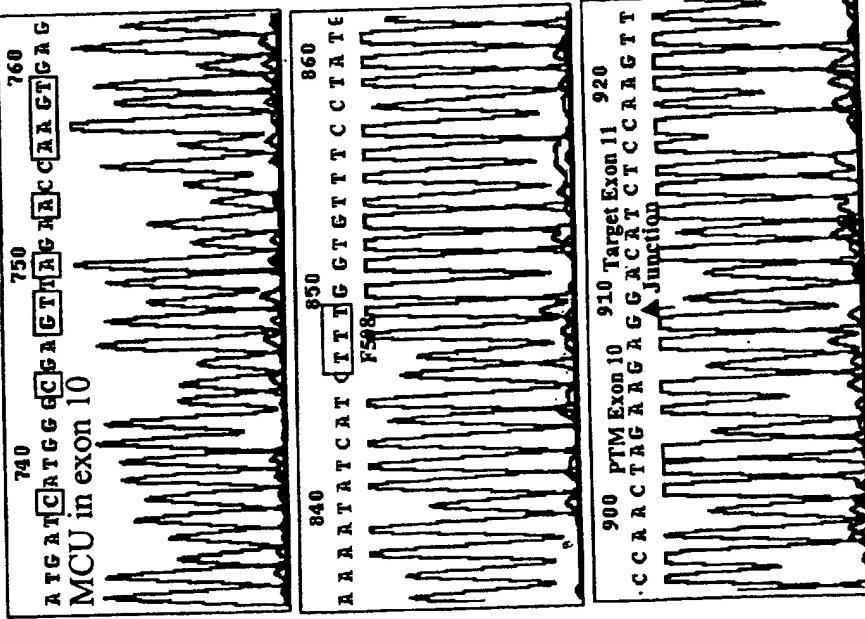
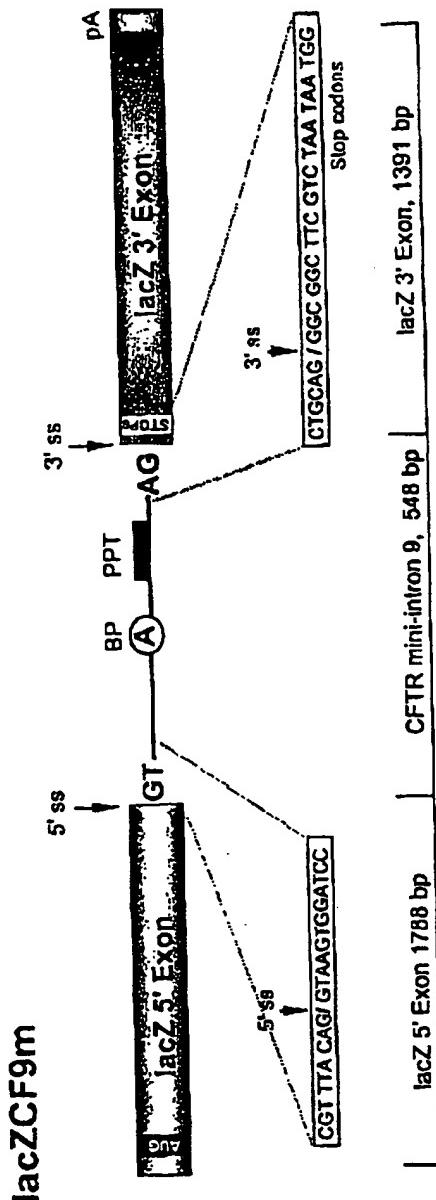


Figure 36

5

25 to 34 myd

Sheet 44 of 58

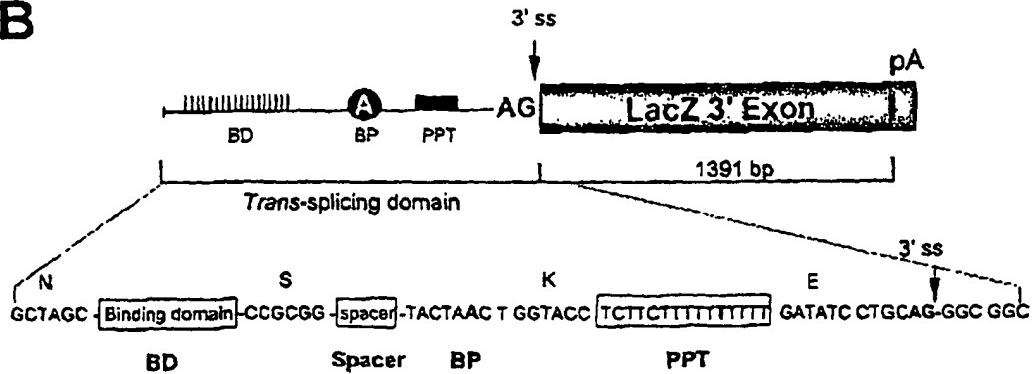


4

Figure 37 A

Sheet 45 of 58

B



lacZCF9m

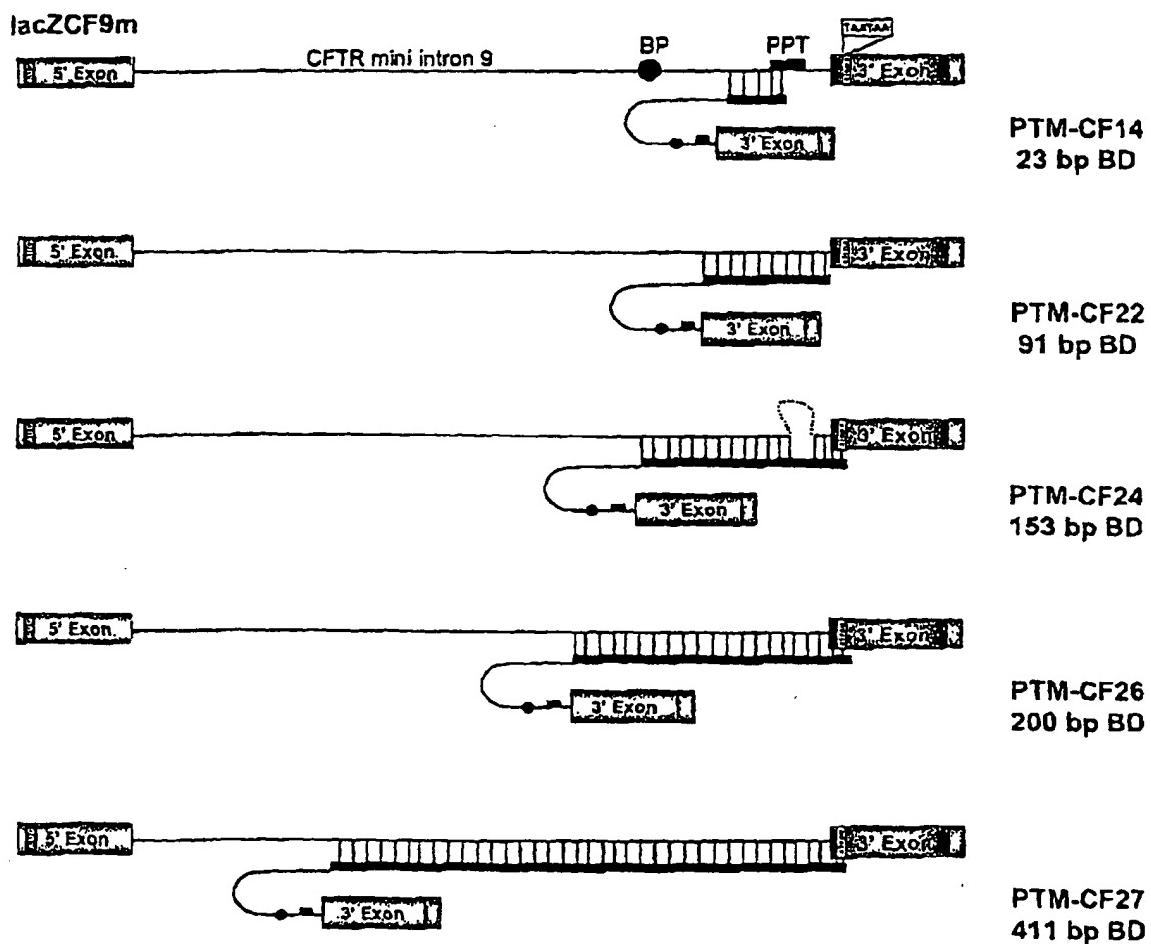


Figure 37B

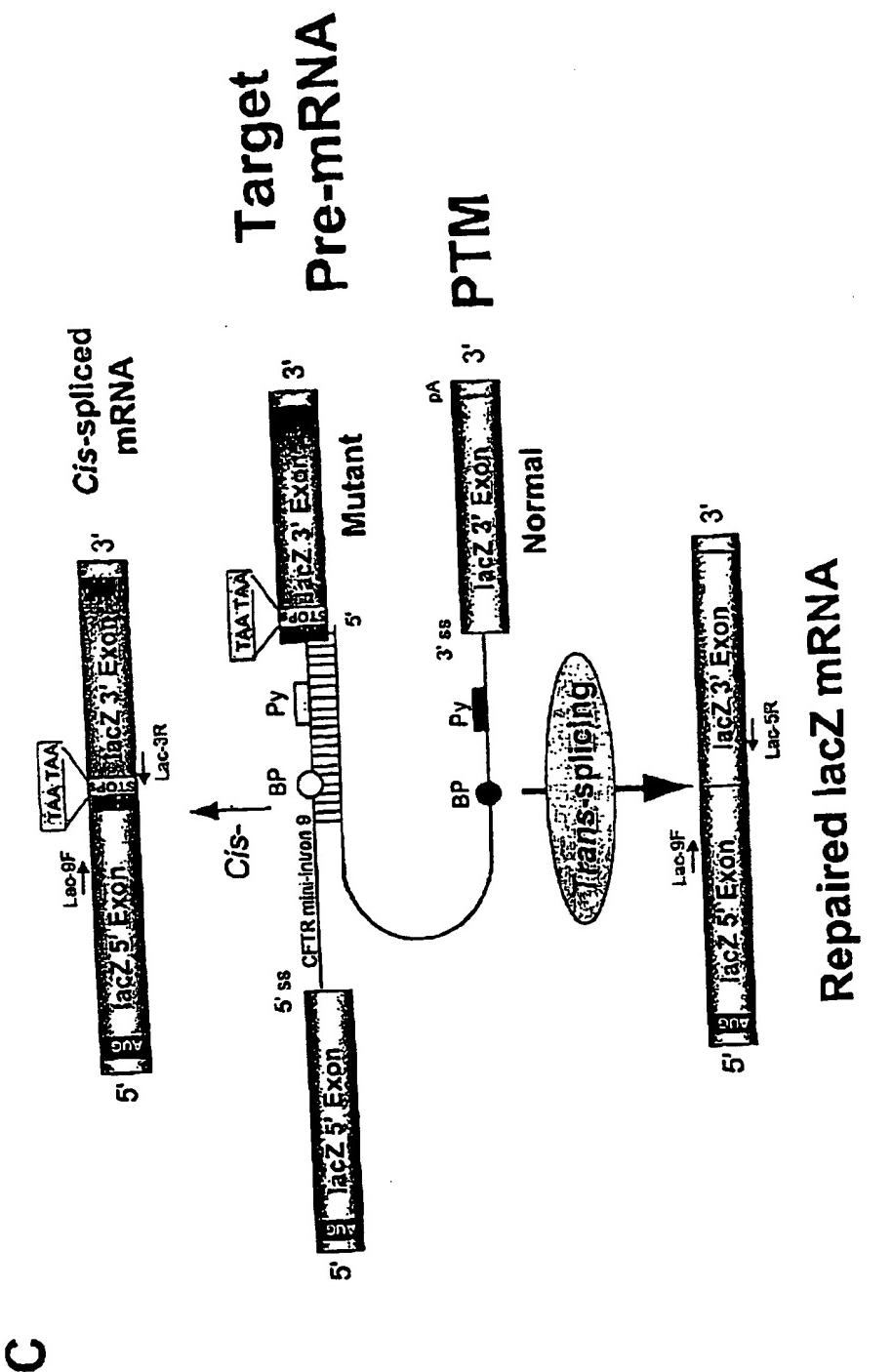


Figure 37C

25 of 46 charts

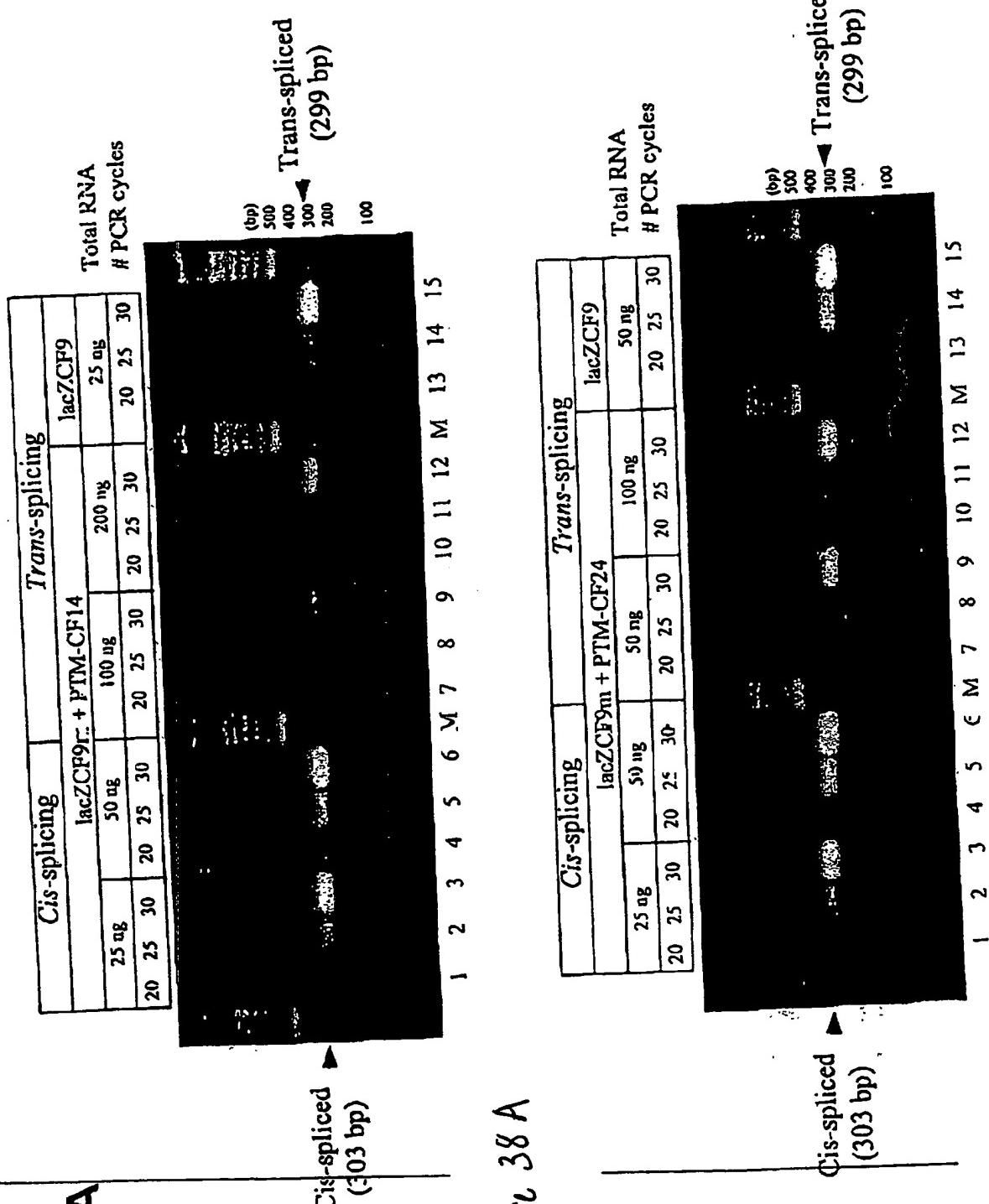


Figure 38A

that 47 my

85

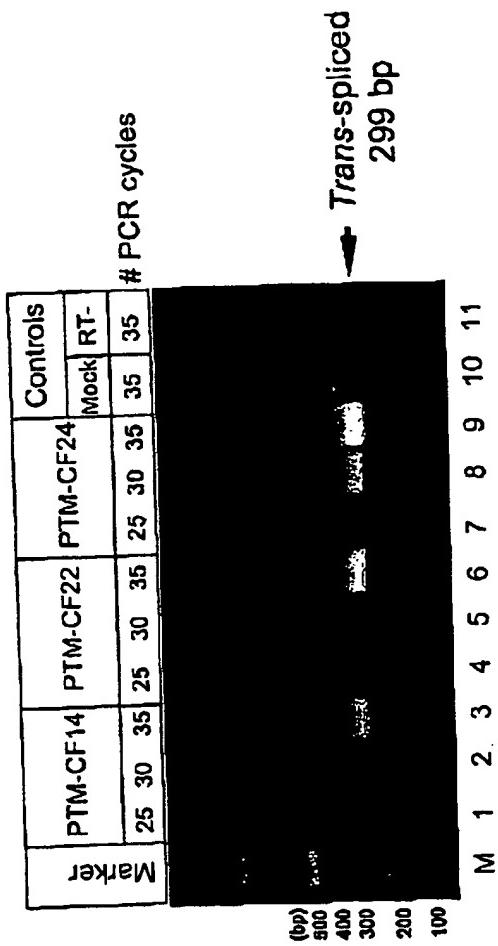
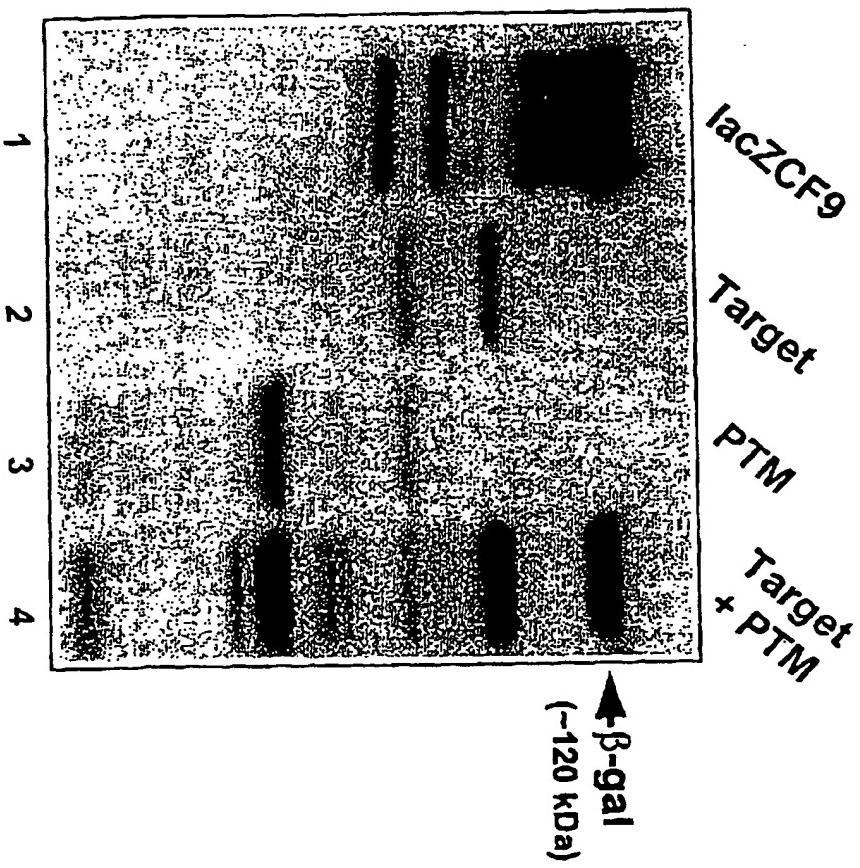
B

Figure 38.B

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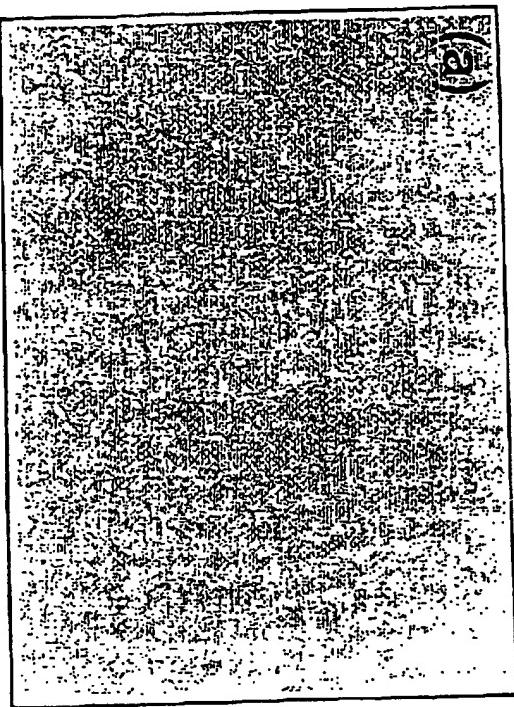
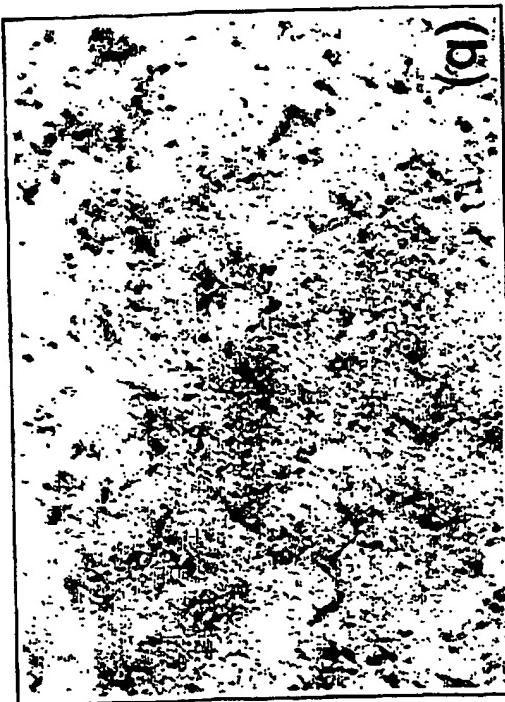
Figure 39



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Figure 40A

A



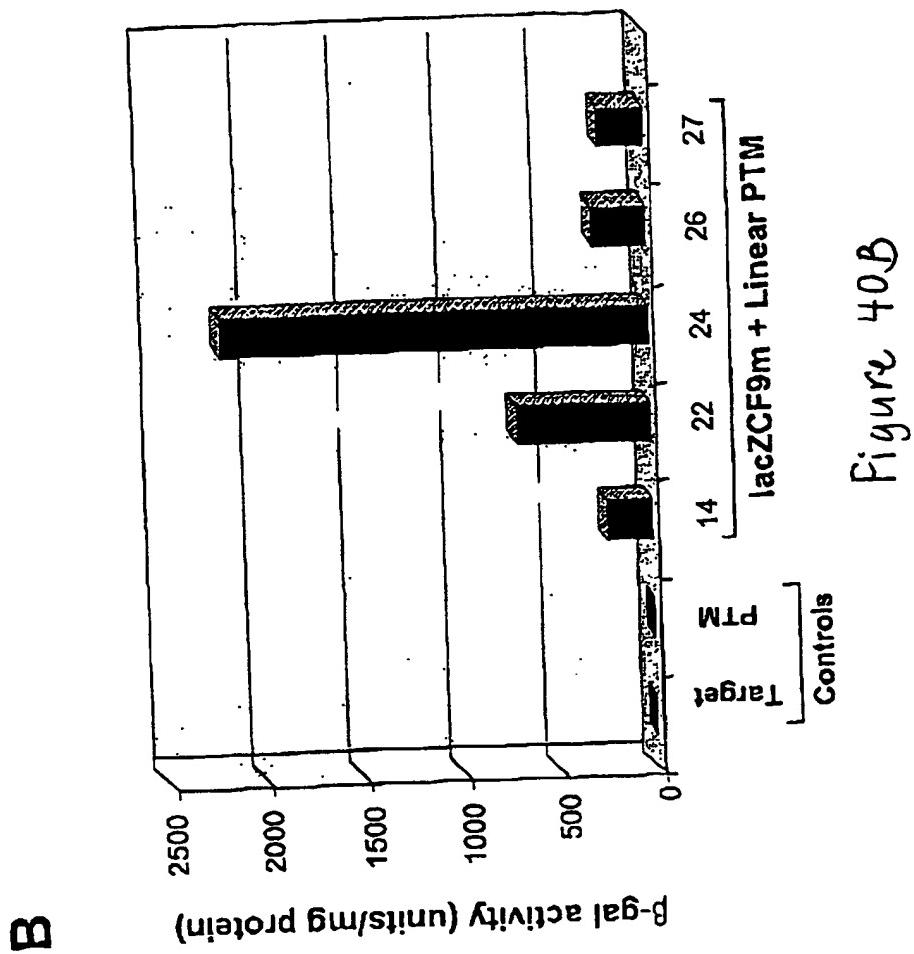
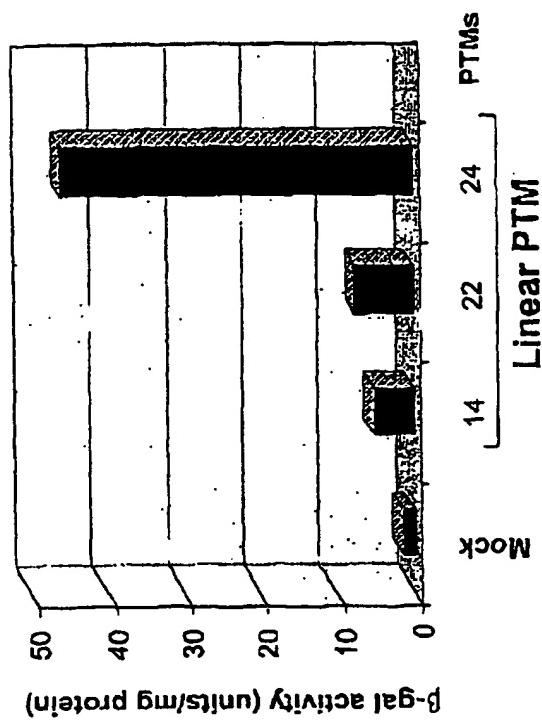


Figure 40B

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C

Figure 40c

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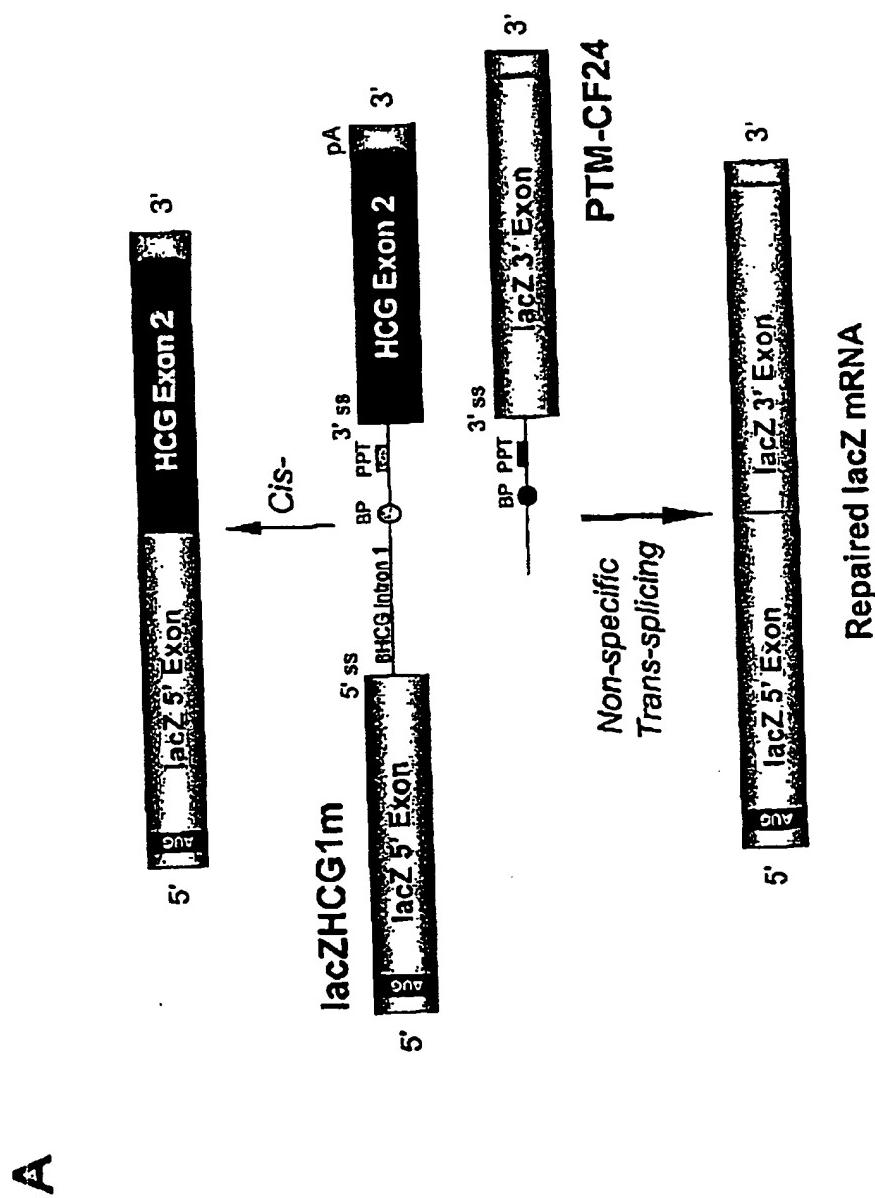


Figure 4IA

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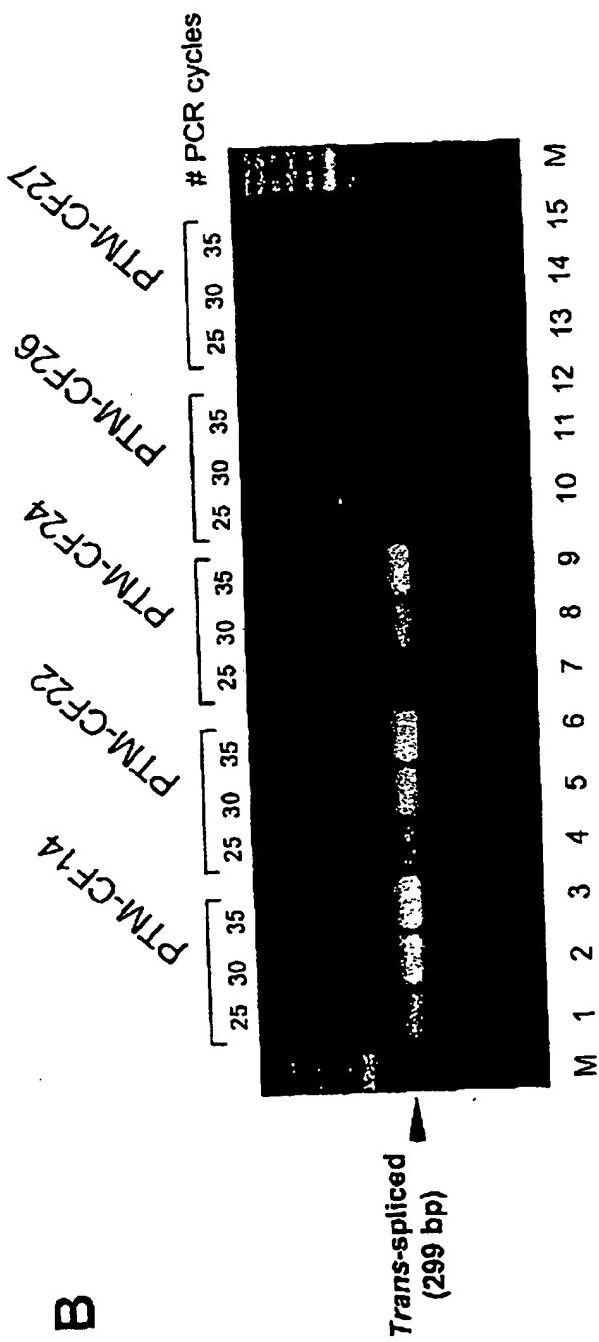


Figure 4rB

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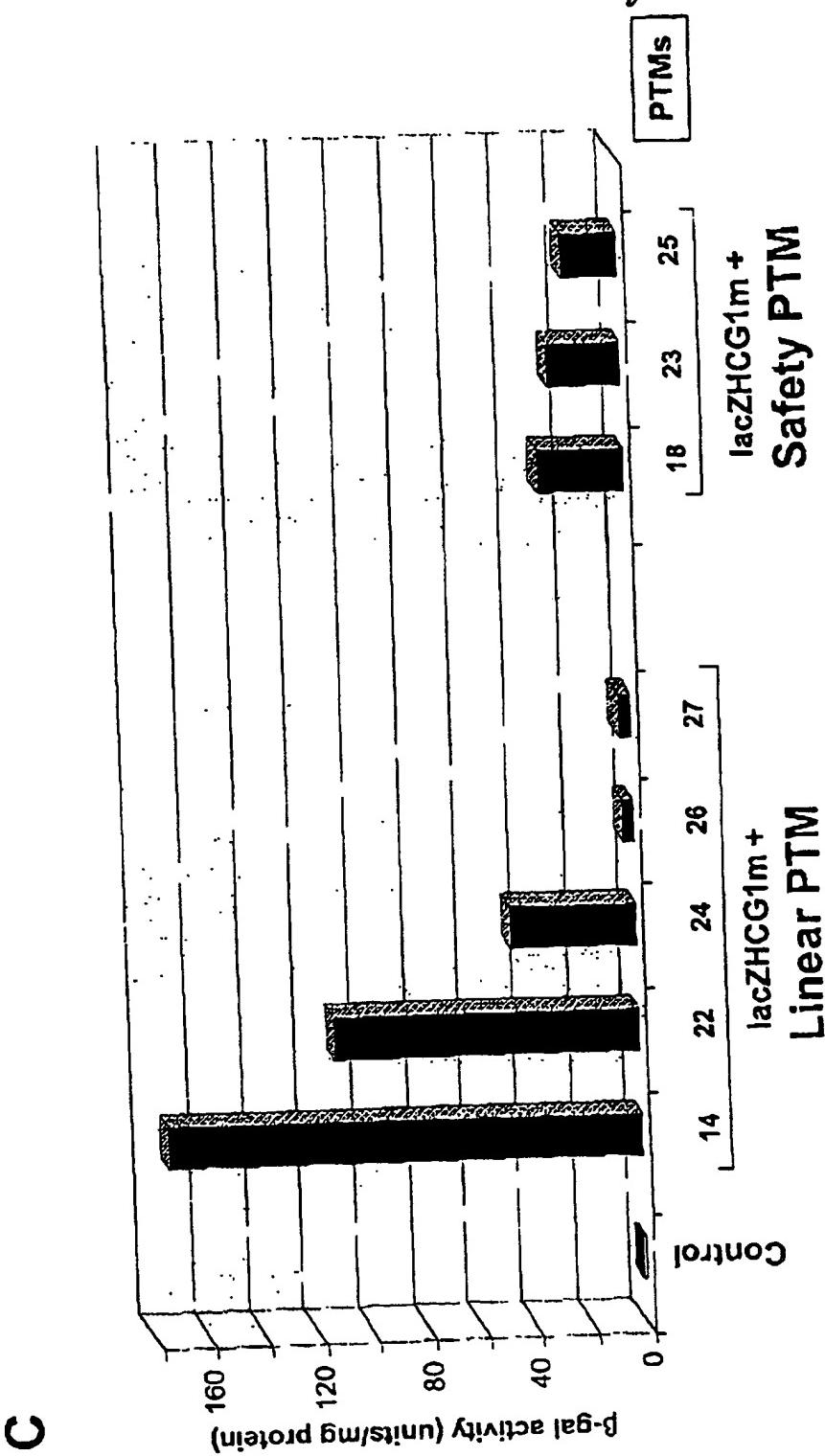


Figure 4C

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Exons 1-10

ATGCAGAGGTGCCCTCTGGAAAAGGCCAGCGTTGTCACAAACTTTTCAGCTGGACCAGACCAATTGAGGAAAG
GATACAGACAGCGCTGGAAATTGTCAGACATAACCAAATCCCTCTGTTGATTCTGCTGACAATCTATCTGAAAATT
GGAAAGAGAATGGGATAGAGAGCTGGCTCAAAGAAAATCTAAACTCATTAATGCCCTCGGCATGTTTCTGG
AGATTTATGTTCTATGGAATCTTTATATTAGGGAAAGTCACCAAAGCAGTACAGGCCCTCTTACTGGGAAGAAC
TAGCTTCCATGACCCGGATAACAGGAGAACCTCTATCGGATTATCTAGGCATAGGCTTATGCCCTCTCTTAT
TGTGAGGACACTGCTCTACACCCAGCCATTGGCCTTCATCACATTGGAAATGAGATGAGAATAGCTATGTTAGT
TTGATTATAAGAACACTTAAAGACTGTCAAGCGCTGTTCTAGATAAAATAAGTATTGGACAATTGTTAGTCTCC
CCAACAACCTGAACAAATTGAGTGAAGGACTTGCATTGGCACATTCTGTGGATCGCTCCCTTGAAGTGGCACTC
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AAATGATCGAGAACATCCAATCTGTTAAGGCATACTGCTGGAGAACAGCAATGGAAAAATGATTGAAAAC
AACAGAACTGAAACTGACTCGAAGGCAGCCTATGTGAGATACTTCAATAGCTCAGCCTCTCTCAGGGTTCTT
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GGAG

Trans-splicing domain

GTAAGATATCACCGATATGTCATAACCTGATTGGGCCCTCGATAACGCTAAGATCCACCGG
TCAAAACTTTACATAATTCTTACCTCTCTGAAATTGATGCTTGTATCTATATTGATCATGTTG
GAAACACCAATGATATTCTTAAATGGTGCCTGGCATAATCTGGAAAATCTGATAACACAATGAAATTCTCCACTGT
GCTTAATTCTACCCCTGAAATTCTCCATTCTCCCATAATCATCATTACAACGACTCTGGAAATAACCCATCATT
ATTAACGCTTACAAATCACGCT

Figure 42

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153 bp PTM24 Binding Domain:

Nhe I 153 bp BD underlined
GCTAGC-GACGAAGCCGCCCTCACGGCTCAGGATTCACTTGCCCTCCAATTAACCTGCTATA

TTCCTTATTGTTAAAGATTCTATAACTCATTTGATTCAAATAATTAAATTTAACCTTCCCTGTTCACCTCTGCTATGC

Sac II
AC-CCGGGG

Figure 43A

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Trans-splicing domain

AATAATGACGAAGCCGCCCTCACGCTCAGGATTCACTGCCCTCCAATTATCATCCTAACAGAAGTGTATATTCTTA
TTTGAAAGATTCTATTAACTCAATTGATTCAAAATTTAAACTTCCCTGTTCACCTACTCTGCTATGCACCCGC
GGAACATTATTATAACGTTGCTGAATACTAACGGTACCTCTTTTTTGTATATCCTGCAG

Exons 10-24

ACTTCACTTCAATGATGATTATGGGAGAACTGGAGCCTTCAGAGGGTAAATTAAAGCACAGTGGAAAGAATTCTATTCT
GTTCTCAGTTTCTGGATTATGCCCTGCCACCAATTAAAGAAAATATCATCTTGGTCTTCTATGATGAATATAGATA
CAGAAGCGTCATCAAAGCATGCCACTAGAAGAGGGACATCTCAAGTTGAGAGAAAGACAATATAGTTCTGGAGAA
GGTGGAAATCACACTGAGTGGAGGTCACAGAGCAAGAATTCTTAGAAGAGCAGTATACAAAGATGCTGATTGTATT
TATTAGACTCTCTTTGGATACCTAGATTTAACAGAAAAAGAAATATTGAAAGCTGTCTGTAACACTGATGGC
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GATCAGCACTGGCCCCACGCTTCAAGGAGGAGCTGTCTGAACCTGATGACACACTCAGTTACCAAGGT
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ACACTCCCTTCAGAACAAACGGAAATAGTACTCATACTAGAATAACAGCTATGCACTGATTATCACCAGCACCAGTTC
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ATTGACTTCATCCAGTTGTTATTAAATTGTGATTGGAGCTATAGCAGTTGTCGAGTTTACAAACCTACATTTGTT
GCAACAGTGCAGTGTAGTGGCTTTATTATGTTGAGAGCATATTCTCCAAACCTCACAGCAACTCAAACAACTGG
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GCAGCCTACTTGAACACTCTTCCACAAAGCTCTGATTTACATACTGCCAACCTGGTTCTGTACCTGTCAACACTG
CGCTGGTCCAAATGAGAATAGAAATGATTTGTCTTCTGCTTACCTTCATTCCATTAAACAACAG
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CAGCATAGATGGATAGTGTGATCTGAGCTTGTGAGCCGAGTCTTAAGTCTATTGACATGCCAACAGAAGGTAACCT
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AACTCTAAACAGATTGCTGATTGCAAGTAATTCTGTGAAACACAGGATAGAAGCAATGCTGAATGCCAACAA
TTTTGGTCAAGAAGAGAACAAAGTGCAGCAACTGCTGAAACAGGATAGAAGCAATGCTGAAGTGGCTTCCGGC
AAGCCATCAGCCCTCCGACAGGGTGAAGCTTTCCCCACCGGAACCTCAAGCAAGTGCAGTCAAGCCCCAGATTGC

Histidine tag Stop

TGCTCTGAAAGAGGAGACAGAAGAGGGTCAAGATACAAGGCTTCATCATCATCATCATTAG

Figure 43B